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M.Sc. Thesis

AN INVESTIGATION OF THE
OXIDATION-REDUCTION POTENTIALS OF
THE METHYLENE BLUE THIOCYANATE SYSTEM

by

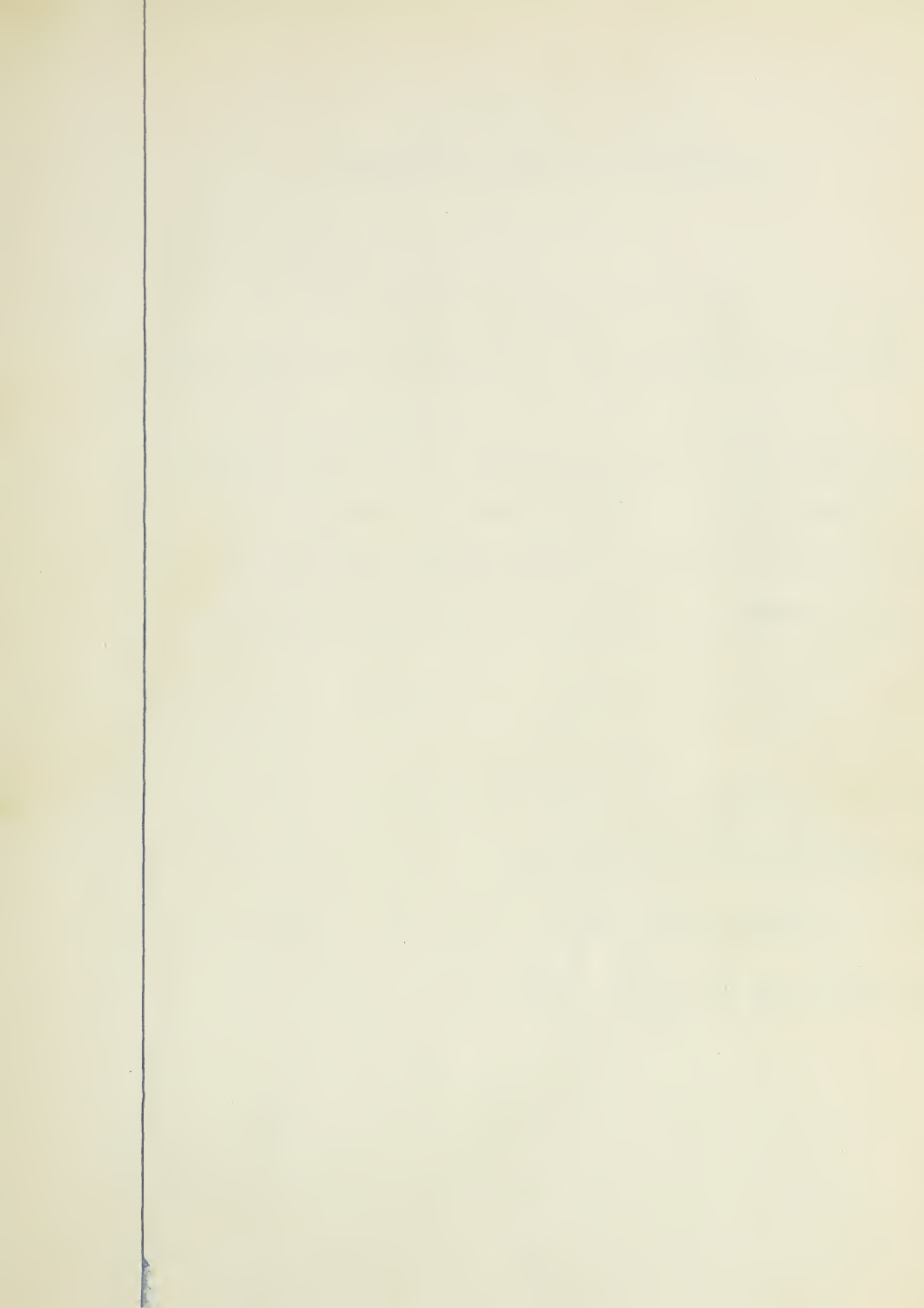
Carl Stinson Miller

Dept. of Chemistry.

April 1936.

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by

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Under the direction of Dr. R.B. Sandin

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A THESIS

Submitted to the Committee on
Graduate Studies, University of
Alberta, in partial fulfilment
of the requirements for the
degree of Master of Science.

Time devoted to thesis work: 5 months out of


14 months devoted to M.Sc. work.

Edmonton, Alberta

April, 1936

ACKNOWLEDGEMENT

The author wishes to express his gratitude to Dr. R. B. Sandin, without whose advice and assistance this work would not have been possible.



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AN INVESTIGATION OF THE OXIDATION-REDUCTION POTENTIALS OF THE METHYLENE BLUE THIOCYANATE SYSTEM

The Object of the Investigation

The object of this investigation was primarily to determine the oxidation-reduction potentials of methylene blue thiocyanate over a complete range of hydrogen ion concentration. It was also desired to compare the results obtained with those of W.M. Clark, who worked with the impure methylene blue chloride, since the thiocyanate can be prepared in a state of excellent purity.

The work is also of importance in connection with the methylene blue reduction test for the bacterial content of milk. It is important to demonstrate that the thiocyanate salt, which may be used in preference to the chloride, in this test, is reduced over the same range of potential as the chloride.

The Methylene Blue Reduction Test

Before commencing on a more detailed study of oxidation reduction, it may be well to first consider the methylene blue reduction test.

The test may be regarded as an index of the care exercised by the milk producer in the handling of the milk. Bacteria in the milk due to their oxygen

requirements, reduce the dye to the leuco form.

Due to the impurity of the methylene blue chloride which was used for the test, the best working standard which was available was, that at the commencement of the test, the methylene blue - milk solution should be of a "robin's egg blue" color. The time required for this color to disappear was an index of the bacterial content of the milk. For example, good milk would take from five to six hours to completely reduce the dye, fair milk from two to five hours, and bad milk less than two.

The advantages of substituting the thiocyanate salt for the chloride, in the test, are few in number but very important.

1. Methylene blue thiocyanate can be prepared practically 100% pure, thus giving the dairyman an absolute standard to work by.

2. It is thought by some workers that due to its purity,^b the thiocyanate gives a clearer blue color and a sharper end point.

(1)

Other substances such as janus green and
(2)

Resazurin have been suggested for use in similar reduction tests.

In this investigation it was found that over a complete range of pH the methylene blue thio-

cyanate gave the same oxidation-reduction potential as the methylene blue chloride. Thus we may assume that from this standpoint it is a suitable substitute for the chloride in the reduction milk test.

Some Theoretical Aspects of Oxidation-Reduction Potentials.

Introduction

Upon that great problem, biological oxidation, the attack has been continuous from 1774, when Joseph Priestly isolated "Dephlogistigated air" and with it conducted animal experiments. In 1775 Lavoisier published his discovery of oxygen, and two years later his studies on respiration gave new meaning to the fact that the life of man hangs, moment to moment, upon the maintenance of a line of communication between his tissues and the air. This knowledge inclined all investigators to state the facts of biological oxidation in terms of the element oxygen itself. The facts of anaerobiosis brought to light by Pasteur have been twisted to fit preconceptions, and Ehrlich's suggestive studies on the reduction of dyes by tissues have been interpreted in terms of oxygen demand. As a consequence of this attitude the main problem has been regarded to be the discovery of the mechanism by which the oxygen of our atmosphere enters

the chemistry of life.

When in 1920 Gillespie published some suggestive data upon electrode potentials induced by bacterial reduction, there was hoped that a method had been revealed, whereby it might be possible to accumulate quantitative data and, step by step, build up exact evidence upon one of the manifold aspects of the general problem.

W.M. Clark repeated Gillespie's work and confirmed its main features, but he came to the conclusion that some of the remarkable relations suggested were altogether too important to justify their acceptance, without more rigid confirmation. There also seemed to be a need for a supplementary method with which to check electrode measurements of reduction intensity.

Accordingly he suspended biological studies and began a study of the potentials of various dyes in equilibrium with their reduction products--systems which can be used as indicators of oxidation-reduction intensity in a manner comparable to the use of acid-base indicators in hydrogen ion studies.

Since the various aspects of the subject are somewhat complicated it seems best to preface the

description of experimental data by a review of elementary principles and a theoretical analysis of relations between electrode potentials and **pH**.

The Meaning of Oxidation-Reduction

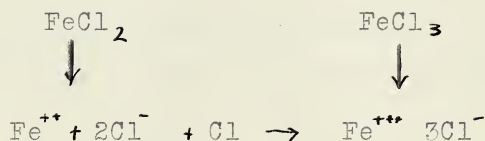
The Element iron is known in aqueous solution in two states, each recognized by specific reactions. The one is called the ferrous, the other the ferric state. Ferrous iron may be converted into ferric iron by any one of a group of reagents, among which are oxygen, chlorine and permanganate. In each case the nature of the change in the state of the iron is the same, even though the transforming agents vary. We then seek the factor common to the three agents. Two of them contain oxygen, and if we consider that chlorine reacts with water to liberate oxygen we may assume that in each case the agent common to the transformation of ferrous to ferric iron is oxygen.

Thus it was that a great many transformations came to be systematized in terms of the participation of oxygen and were termed "Oxidations". In the progressive action of oxygen the products were known as higher and higher states of oxidation. The reverse process was then looked upon as leading to lower states and was termed "reduction." This

term however, was applied not only to the removal of oxygen but also to the addition of hydrogen. This is of no serious consequence to the systematist since he can always devise a way to show that the addition of hydrogen is equivalent to the removal of oxygen. Given the liberty of avoiding a roundabout expression of experimental facts the experimentalist will avoid any consideration of oxygen in describing a reaction such as



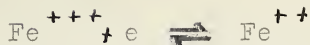
As a matter of fact, the best evidence indicates that such salts exist largely in the ionized forms, as indicated below, and the reaction may be regarded as the transference of an electron.



That is $\text{Fe}^{++} \longrightarrow \text{Fe}^{+++}$ plus an electron. The chlorine is thus merely the absorbent of electrons, and we can conceive of any agent which induces the reaction, to act merely by withdrawing electrons to itself. The reverse process, the transformation of Fe^{+++} to Fe^{++} , could be conceived as due to any agent which contributes electrons to Fe^{+++} .

The case under discussion is a reversible

reaction which may be expressed by means of double arrows,



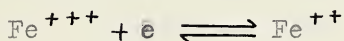
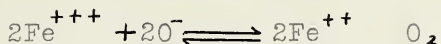
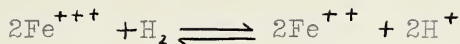
Thus the breach in the original systematic classification has been widened until there has entered a meaning quite foreign to the original terms "oxidation" and "reduction". If required to define terms we can say that oxidation can be regarded as the withdrawal of electrons from a substance, with or without the addition of oxygen or elements analagous to oxygen or as the withdrawal of electrons, with or without the withdrawal of hydrogen or elements analagous to hydrogen. Reduction is the reverse of oxidation as defined above.

Oxidation Reduction Equilibria

Since the ion Fe^{++} is capable of losing an electron it may be regarded as a reducing agent. Since Fe^{+++} is capable of taking up an electron it may be regarded as an oxidizing agent. Now it is practically impossible to prepare a solution absolutely pure with respect to either oxidant or reductant and in many instances it is desirable to know the degree to which a mixture of oxidant and reductant is oxidized or reduced by another system. Our interest then centers on the ratio of reductant to oxidant

and upon the conditions under which this ratio assumes different values.

As suggested in the previous section we can regard the reversible transformation of ferric to ferrous iron to proceed through any one of a number of possible courses such as the following:



If we express the concentrations by means of brackets, the equation expressing the equilibrium conditions for the cases mentioned are as follows:

$$\frac{(\text{Fe}^{+++})^2 (\text{H}_2)}{(\text{Fe}^{++})^2 (\text{H}^+)^2} = K_1 \quad \text{or} \quad \frac{(\text{Fe}^{+++})}{(\text{Fe}^{++})} = \sqrt{\frac{K_1 (\text{H}^+)^2}{(\text{H}_2)}}$$

$$\frac{(\text{Fe}^{+++})^2 (\text{O}^-)^2}{(\text{Fe}^{++})^2 (\text{O}_2)} = K_2 \quad \text{or} \quad \frac{(\text{Fe}^{+++})}{(\text{Fe}^{++})} = \sqrt{\frac{K_2 (\text{O}_2)}{(\text{O}^-)^2}}$$

$$\frac{(\text{Fe}^{+++}) (e)}{(\text{Fe}^{++})} = K_3 \quad \text{or} \quad \frac{(\text{Fe}^{+++})}{(\text{Fe}^{++})} = \frac{K_3}{(e)}$$

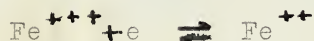
For any given ratio of $\frac{(\text{Fe}^{+++})}{(\text{Fe}^{++})}$,

$$\sqrt{\frac{K_1 (\text{H}^+)^2}{(\text{H}_2)}} = \sqrt{\frac{K_2 (\text{O}_2)}{(\text{O}^-)^2}} = \frac{K_3}{(e)}$$

This procedure is capable of indefinite

expansion and shows that from the schematic point of view we are at liberty to choose any hypothetical scheme with which to express the equilibrium state. The discovery of finite values for the chosen terms does not prove that the selected species are alone involved, in the actual mechanism, and that values representing no actuality are still useful for purposes of calculation.

It will be found convenient to use the following schematic treatment. In a solution containing ferrous and ferric ions there can be a continuous exchange of electrons as represented by the reversible reaction.



It is improbable that the mechanism of this exchange is such that there are present at any instant an appreciable number of free electrons. On the other hand the solution certainly possesses the ability to "reduce" certain substances brought in contact with it. This ability is expressed in terms of the electron escaping tendency, or "fugacity." We shall not do great violence to the subject if in this discussion we replace the "fugacity" by the more familiar term "concentration" and formulate the equilibrium state of the reaction in accordance with

the mass law as follows:

$$\frac{(F^{+++}) (e)}{(Fe^{++})} = K$$

In general for any oxidation-reduction reaction, involving the transfer of n electrons, the equilibrium state is formulated by

$$\frac{(Ox) (e)^n}{(Red)} = K$$

Where (Ox) represents the active concentrations of oxidant and (Red) represents the active concentration of reductant.

Now let the equilibrium of one system be formulated by $\frac{(Red)}{(Ox)} = \frac{(e)}{K}$

and those of another system be formulated by

$$\frac{(Red')}{(Ox')} = \frac{(e)}{K'}$$

Since (e) is now common to both equilibria

$$\frac{(Red)}{(Ox)} = \frac{K'(Red')}{K(Ox')}$$

If the absolute, or relative values of K and K' were known we could at once predict what ratio of given oxidant and its reduction product would exist with a given ratio of another oxidant and its reduction product. For instance, if $\frac{(Red)}{(Ox)}$ 1, the relation $\frac{(Red')}{(Ox')}$

$\frac{K'}{K}$ determines whether the second system is practically completely reduced, practically completely oxidized or at some intermediate stage. This would enable us to arrange all systems on a relative oxidation-reduction scale. The position of a system on such a scale would show its oxidizing or reducing tendency with respect to other systems.

Electrode Potentials

An evidence of some actual state of which (e) is representative is found in the fact that a noble metal placed in a solution containing an oxidation-reduction system, acquires an electron charge increasing in intensity with increase of the reducing tendency, the electron fugacity, or as we choose to say, the (e) value of the solution.

Let us assign the value (e_m) to the concentration of free electrons in the noble metal.

In transferring isothermally one faraday of electrons from concentration (e_m) to (e_s) , ((e_s) is the electron concentration in the solution), the work done is W .

$$W = RT \ln \left(\frac{e_m}{e_s} \right)$$

Where R is the gas constant and T the absolute temperature.

Since $W = E F$ for this reaction, where E

the electromotive force, and F the faraday, then

$$E = \frac{RT}{F} \ln (e_m) - \frac{RT}{F} \ln (e_s)$$

Considering (e_m) to be constant

$$E = C' - \frac{RT}{F} \ln (e_s)$$

Now writing the equilibrium reaction for any oxidation-reduction reaction

$$\frac{(Ox) (e)^m}{(Red)} = K$$

$$\text{then } (e) = \sqrt[m]{K \frac{(red)}{(Ox)}}$$

Substituting this in our equation

$$E = C' - \frac{RT}{nF} \ln K \frac{(Red)}{(Ox)} = C' - \frac{RT}{nF} \ln K - \frac{RT}{nF} \ln \frac{(Red)}{(Ox)}$$

If we know n and the ratio $\frac{(Red)}{(Ox)}$ for two systems, the values of

$$C' - \frac{RT}{nF} \ln K_1$$

and $C' - \frac{RT}{nF} \ln K_2$ could be found.

Their difference being

$$\frac{RT}{nF} \ln \frac{K_1}{K_2} \text{ from which we may}$$

determine the value of $\frac{K_1}{K_2}$. This ratio, as we

have already seen, permits the arrangement of the various systems in their relative position on a scale.

If now we measure such electromotive forces against a hydrogen electrode, taking the single electrode potential of the hydrogen electrode as zero, we have a set of readings of E , in which E is defined as the difference in potential between the electrode and the normal hydrogen electrode. To make this clear, it is written E_h . Since the constant C is expressed in volts it will be written E_o . This includes all factors in the cell, contributing to the E.M.F. except (Red) and (Ox)

$$E_h = E_o - \frac{RT}{nF} \ln \frac{(\text{Red})}{(\text{Ox})}$$

We have now arrived at a result amenable to experimental test, and which has been successfully submitted to such tests in a wide variety of instances.

When $\frac{(\text{Red})}{(\text{Ox})} = 1$, $E_h = E_o$. If then it is possible to fix $\frac{(\text{Red})}{(\text{Ox})}$ and to determine experimentally, definite E_h values in any given system, it is possible to express "relative oxidation-reduction intensities in terms of electrode potential!" This is illustrated graphically in figure 1, where E values are plotted as ordinates and instead of the ratio $(\text{Red}) / (\text{Ox})$, the percentage reduction is plotted as abscissa. The distinctly different slopes of A

and B are determined by the value of n in the equation. It will also be noted that the position of a system in the potential scale depends on the value of E_o and within any one system E_h depends on the ratio of reductant to oxidant and not on the total concentration.

It must be borne in mind that E_h is a measure of oxidation-reduction intensity and not capacity. It will be noted that when $(Red) / (Ox)$ approaches one, the value of E_h is stabilized. This is comparable to buffer action and is known as "poising action."

The charting of all systems as in figure 1, would then systematize the subject thoroughly, were it not for difficulties which we will now have to deal with.

Theoretical Relations Between Reduction Potentials

and pH

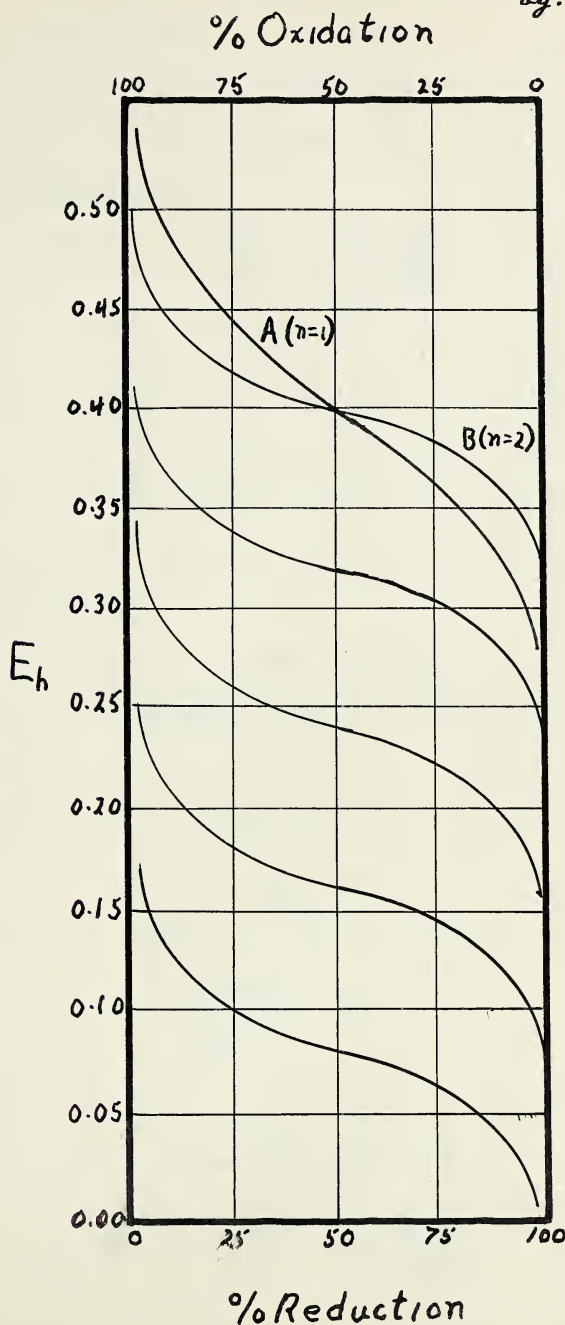
We have already shown that for the reaction $Ox + ne \rightleftharpoons Red$, the E.M.F. of such a system measured against a hydrogen electrode is

$$E = C - \frac{RT}{nF} \ln \frac{(Red)}{(Ox)}$$

It must now be emphasized that to be consistent we must identify the reductant as an anion

Figure I

Curves showing the variation
of E_0 from one system to another.
By. W. M. Clark.



and the oxidant as a cation. In two simple cases we would then have

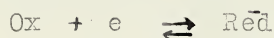


For the first case

$$E = C - \frac{RT}{nF} \ln \frac{(\text{Red})}{(\text{Ox})}$$

Here the active reductant is the anion of an acid and its concentration is therefore dependent not only on the total amount of reductant present, but also on conditions which govern ionization. "Among these conditions the hydrogen ion concentration is most important."

Since it is often difficult to obtain the exact values of (Red) it will be more useful to set up equations relating E to total reductant present and total oxidant. Assuming our reaction to be



the equilibrium constant can be represented

$$\frac{(\text{Ox})(e)}{(\text{Red})} = K$$

Combining this with the fundamental electrode equation

$$E = C' - \frac{RT}{F} \ln (e)$$

$$E_h = C - \frac{RT}{F} \ln \frac{(\text{Red})}{(\text{Ox})}$$

In dilute solution we may assume that the total reductant (S_r) is the sum of the anions and non-ionized acid. That is

$$(S_r) = (H \text{ Red}) + (Red^-)$$

The equilibrium equation for the acid dissociation of the reductant

$$\frac{(Red^-) (H^+)}{(H \text{ Red})} = K_a$$

Considering these two equations we get that

$$(Red^-) = \frac{(S_r) K_a}{(H^+) + K_a}$$

Substituting this in the E.M.F. equation we get

$$E_h = E_o - \frac{RT}{F} \ln \frac{(S_r)}{(S_o)} - \frac{RT}{F} \ln \frac{K_a}{(H^+) + K_a}$$

Here (S_o) represents the total concentration of oxidants.

Inspection of this equation shows that when the ratio (S_r) / (S_o) is kept constant, E_h will vary with pH alone. When K_a is small it will be a linear function of pH and when K_a is large the variation will be inappreciable. When $(H^+) = K_a$, E_h will be the mid point of the inflection, connecting the one extreme set of conditions with the other. This fact makes K_a determinable experimentally.

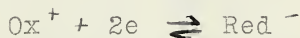
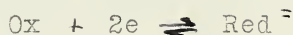
If on the other hand the hydrogen ion concentration is held constant by means of buffers we can write the constants then found, and the equation becomes

$$E_h = E_o' - \frac{RT}{F} \ln \left(\frac{S_a}{S_b} \right)$$

This equation is identical with the one obtained before. Now, however, we are working with measurable quantities of oxidant and reductant.

If the value of E_h is known for each value of the ratio $\left(\frac{S_a}{S_b} \right)$ the number of electrons concerned is determinable. This of course, is fundamental in the investigation of any new system.

If we have two electrons to deal with there are several possibilities. Three will be indicated at this point.



In dealing with any one of these we would treat exactly as before and would arrive at an equation of the form

$$E_h = \text{constant} - \frac{RT}{nF} \ln \left(\frac{S_a}{S_b} \right) - \frac{RT}{nF} \ln \left[\begin{array}{l} \text{function of} \\ (H^+) \text{ and} \\ \text{equilib. const.} \end{array} \right]$$

In this investigation we are primarily interested in the potentials of the methylene blue

system, thus we will terminate our general discussion and enter upon a more specific discussion.

The Electrode Equation of a Thiazine

Both methylene blue and Lauth's violet are thiazines of the same general structure. Lauth's violet contains four less methyl groups than methylene blue.

If for each of these two substances a series of potential readings are made for various values of pH , in each case keeping the ratio $\frac{(S_1)}{(S_2)} = 1$ and the results plotted, as E_A against (S_2)

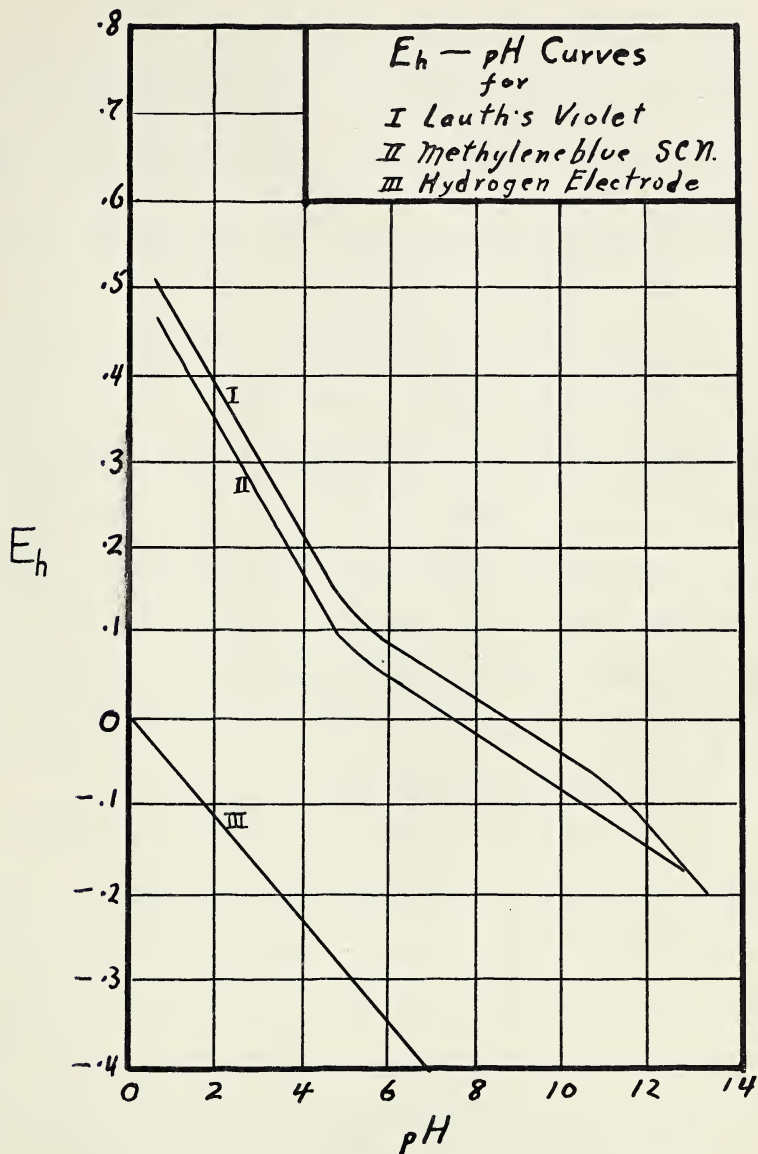
pH , we would obtain curves such as are shown in figure 2.

Then with the experimental data before us we come to their formulation in accordance with the principles outlined before.

Since the Lauth's violet system displays an inflection in the $E_A - pH$ curve (figure 2), in alkaline solutions which the methylene blue system does not, it furnishes the more complete picture. Therefore, the following interpretation will be made with the aid of Lauth's violet.

In the acid region, the value of $\frac{-dE}{dpH}$ is

Figure 2



Taken from the work of W. M. Clark.³

0.09 which we shall call the 0.09 slope. The 0.09 slope abruptly changes to 0.03 slope at a pH of about 5, and since two electrons or their equivalent are concerned in the reduction process making the $\frac{RT}{nF}$ coefficient 0.03, this change of 0.06 (i.e. 2×0.03) indicated that two acid base dissociations are encountered in this pH region. The two dissociation constants concerned are obviously not identical, because the actual inflection of the curve is not nearly so abrupt as would be the case were they identical. Do both of these constants represent ionizable groups created or destroyed in the act of reduction? If they do, we still have, unaccounted for, a third group made apparent by the change from a 0.03 slope to a 0.06 slope at pH 11.

Since the electrometric data reveal "directly" little, regarding the nature or the location of the acid base groups encountered, it is possible to express the experimental data as described before; but such equations call for bizarre chemical properties on the part of the thiazines. By adopting the following rational development we can reach a result which seems satisfactory from every viewpoint.

We shall assume that Bernthsen's formulae for a thiazine is essentially correct. Writing

the formula from the electronic point of view we then have for a thiazine formula 1 and for its reductant formula 11 in figure 3.

It will be particularly noted that the double bonded terminal nitrogen, formula 1, contributes but four electrons to the surrounding octet while it has five positive charges reserved for its outer shell. Consequently this group has a distinct polar valence comparable with that of ammonium. On reduction this polar valence is destroyed and at the same time a potential anion is created at the bridging nitrogen.

The oxidation-reduction process may therefore be expressed in the following form

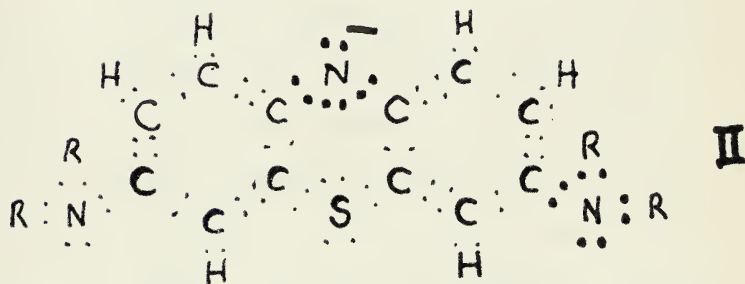
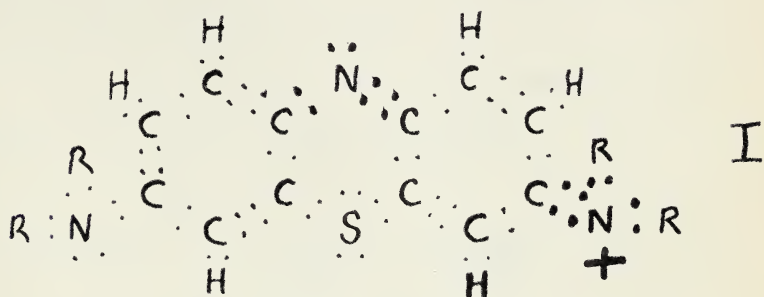


and the corresponding electrode equation is

$$E_h = C - \frac{RT}{2F} \ln \frac{(Red^-)}{(Ox^+)}$$

Next, in summing the various species of oxidant and reductant, to obtain the equation embodying total oxidant (S_o) and total reductant (S_r) we shall have to take into consideration the experimental fact that three changes in steps of 0.03 are found in the slopes of the $E_h : pH$ curve indicating that three dissociation constants are to be considered.

Figure III



the same species. For the reductant the sum (S_n) of all the species is,

$$(S_n) = (\text{Red}^-) + (\text{H Red}) + (\text{H}_2\text{Red}^+) + (\text{H}_3\text{Red}^{++})$$

$$\frac{(\text{Red}^-)(\text{H}^+)}{(\text{H Red})} = K_{n_1}$$

$$\frac{(\text{H Red})(\text{H}^+)}{(\text{H}_2\text{Red}^+)} = K_{n_2}$$

$$\frac{(\text{H}_2\text{Red}^+)(\text{H}^+)}{(\text{H}_3\text{Red}^{++})} = K_{n_3}$$

In the oxidant the group $\text{C}=\text{NH}$ can be brought into Bronsted's formalistic scheme, but it is more realistic to treat it as a cation adding the hydroxyl ion directly. Hence.

$$(S_o) = (\text{Ox}^+) + (\text{Ox OH})$$

$$\frac{(\text{Ox}^+)(\text{OH}^-)}{(\text{Ox OH})} = K_{ob}$$

or since we wish to use (H^+)

$$\frac{(\text{Ox}^+)}{(\text{H}^+)(\text{Ox OH})} K_w = K_{ob}$$

Solving all these equations for (Ox) and (Red) and substituting in the E.M.F. equation and collecting constants we then have,

$$E_h = E_o - 0.03 \log \frac{(S_n)}{(S_o)} - 0.03 \log \frac{K_{ob}(\text{H}^+) + K_w}{K_{n_1}K_{n_2}K_{n_3}(\text{H}^+) + K_{n_2} + K_{n_3}[(\text{H}^+)^2 + K_{n_3}(\text{H}^+)^3 + (\text{H}^+)^4]}$$

Without further discussion we shall assume that the bridging nitrogen fixes the H . Consequently K_{n_1} has a value so low that the terms in which it occurs can be neglected, and we get,

$$E_h = E_0 - 0.03 \log \left(\frac{S_1}{S_0} \right) - 0.03 \log \frac{K_{ob}(H^+) + K_w}{K_{n_2}K_{n_3}(H^+)^2 + K_{n_3}(H^+)^3 + (H^+)^4}$$

In the construction of the E_h : pH curves the equations used were such that $\frac{(S_1)}{(S_0)} = 1$

We also assumed that no essential change would occur when $\frac{(S_1)}{(S_0)} = \frac{\text{normal}}{\text{normal}}$. Thus the values of

E found with dilute solutions can be called the normal potentials.

In the present instance from the last equation when $(H^+) = 1$ and $(S_1)/(S_0) = 1$

$$E'_h = E_0 - 0.03 \log K_{ob}$$

The peculiarity arises from the fact that we have assumed both hydroxyl and hydrogen ions to be concerned, and obviously we would have met a similar situation, had we continued with the same assumption and chosen to formulate the equation in terms of hydroxyl ions.

Dissociation Constants

Since we ascribe a polar valence to the double bonded terminal nitrogen, and discover in Lauth's violet an inflection of the E_b -pH curve at pH 11, we shall give to $\frac{K_w}{K_{ob}}$ a value of 10^{-11} . Tentatively accepting the value 1.88×10^{-14} for K_w at 30° we find K_{ob} to be 1.88×10^{-3}

It will have been noted that we have left out of consideration a second group of potentially basic properties in the oxidant. Were this group active, forming the cation O_2H^{++} within the experimental range of pH, we would have found at some pH zone lower than that at which the 0.03 slope occurs an inflection of the curve tending toward a zero slope. The inflections observed are in the opposite direction. Consequently, we can conclude that the basicity of the amino group in the oxidant is so "weak" that for all practical purposes we may neglect it, with the resulting simplification of the equation. The inflections found, must then be ascribed to ionizations, of the two remaining groups of the reductant. The constants of these two groups are represented by K_{n_2} and K_{n_3}

In determining the values of K_{n_2} and K_{n_3} it is helpful to use the intersection of the pro-

jections of the so-called 0.09 and 0.03 slopes. Those sections of the curve which are found in the regions concerned are, when considered independently, determined by the following equations.

$$-E_1 = 0.03 \log \frac{1}{K_{n_2} K_{n_3}} + 0.03 \rho H - C$$

$$-E_2 = 0.06 \log \frac{1}{K_{n_2} K_{n_3}} + 0.06 \rho H - C$$

$$-E_3 = 0.09 \rho H - C$$

The first of these three determines the 0.03 slope, the last, the 0.09 slope, while the second determines the 0.06 slope between these two limbs of the curve.

The intersection of the 0.03 and 0.09 slopes occurs at $E_1 = E_3$ or when $\log \frac{1}{K_{n_2} K_{n_3}} = 2 \times \rho H$. Graphically we estimate the intersection to be at about ρH 4.9 (figure 2). Hence,

$$\log \frac{1}{K_{n_2}} + \log \frac{1}{K_{n_3}} = 9.8$$

By subsequent trial we find that $\log \frac{1}{K_{n_2}} = 5.3$

and $\log \frac{1}{K_{n_3}} = 4.38$ (sum 9.68, intersection 4.84)

fit the data fairly well. Hence we shall use $K_{n_2} = 5 \times 10^{-6}$ and $K_{n_3} = 4.2 \times 10^{-5}$

It was suggested above that the section of the E_h - ρH curve having a slope of 0.09 lies between

the region of ionization of a group in the oxidant so weak that the K_b value is negligible, and the region of an appreciable ionization of that same group as it appears in the reductant.

Turning now from Lauth's violet to methylene blue, we can apply the same principles, and with the exception of new values for the constants employed, the only essential difference is the absence of an inflection of the curve in the alkaline region. This simply means that in methylene blue the value of K_{ob} is too large to permit suppression of its basic ionization by the alkaline buffers employed.

Discussion of Experimental Methods

In all the experimental work, the concentration of the solutions used was of the order of 0.0001 M. The work was done on the assumption that the observed potentials were dependent on the ratio,

$$\frac{(\text{Red})}{(\text{Ox})},$$

and not on total concentrations of the two together. The work of W. M. Clark (7) has shown that this is not true in the extreme. Thus while there may have been variations in concentration from one experiment to another, all concentrations were of the same order and the authors feel that no error has been introduced, through this source.

In stating results, we shall reduce the single observed potential differences to the hydrogen standard, designating by E_h the reduction electrode potential. In this treatment there are involved, the following assumptions. The ultimate working standard, with which the saturated KCl calomel half cells were compared, was the hydrogen electrode potential difference of an M/20 K acid phthalate solution, to which was assigned the value of - 0.2347 at 25°C. Liquid junctions were in all cases, made with saturated KCl and the

potential differences of such junctions were ignored.

The buffers used in the experimental work were either those suggested by W. M. Clark in his "Studies on Oxidation-Reduction", or ones which were very slightly modified to include citric acid.

Values of pH were run on each buffer just before use. This was necessary, because it was found difficult to accurately duplicate any pH , by simply making up a similar buffer.

All E.M.F. measurements were made at $25^{\circ}C \pm 0.10.^{\circ}$

Oxidation-reduction potentials were measured by three different methods. While only one of these methods gave satisfactory results, all three will be described here, because the author feels that these can be used satisfactorily, and intend to try to accomplish this at a later date.

The first, and from a theoretical standpoint, the best method employed was that of simply measuring the potentials of mixtures of oxidized and reduced forms containing no other oxidizing or reducing substances.

A diagram of the apparatus is shown in

Figure 4

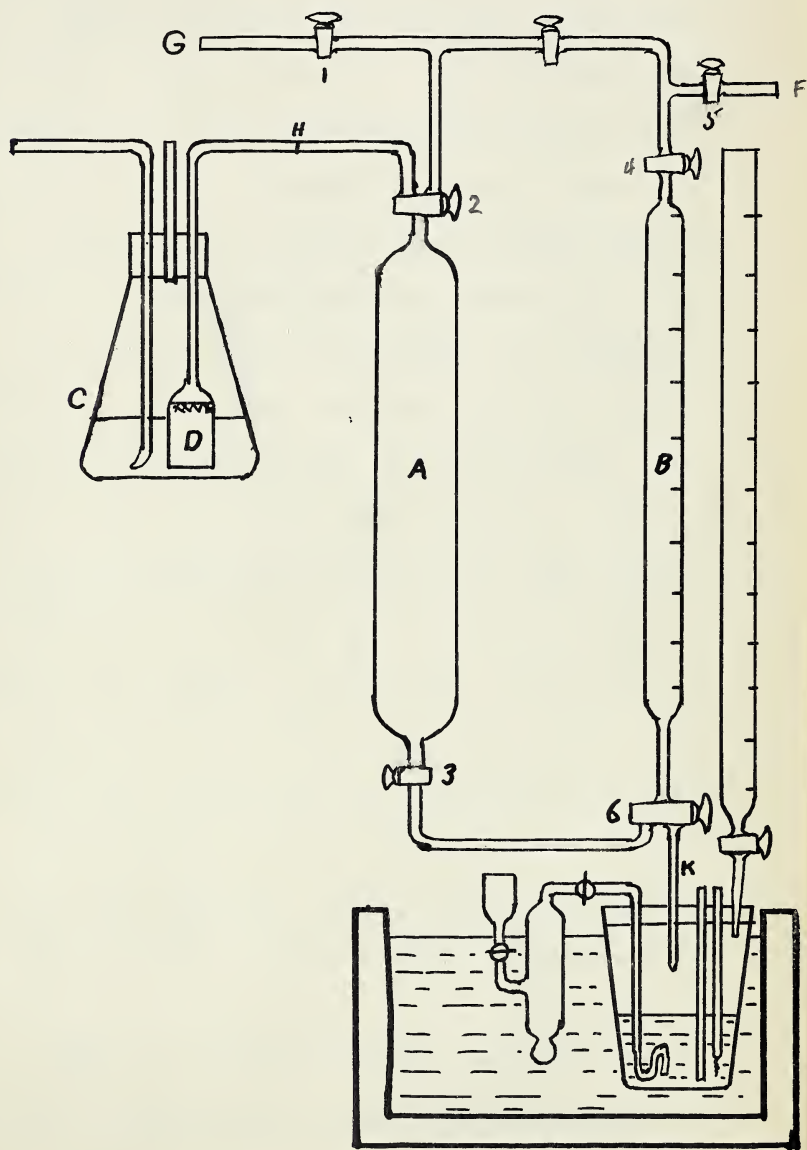


figure 4. This is merely a protected system in which the reduced methylene blue can be prepared, stored, and delivered in measured amounts without coming in contact with oxygen.

When a reduced solution was to be prepared, A was first left empty. Purified nitrogen was passed in at F and different branches of the system were flushed. Meanwhile filter D was prepared. A firm pad of asbestos was formed against the perforated platinum disc and sucked free from water, in order to open its texture for passage of nitrogen. The filter was then attached to H, while nitrogen was escaping. With nitrogen running through the filter, vessel C was put in place containing buffered dye solution and platinized asbestos. The dye was then reduced in C by means of a stream of hydrogen. When the reduction was complete a Cenco Pump was attached at G, cock (5) was closed and the apparatus was evacuated. Cock (1) was opened only when an outward flow of gas was insured. Cock (2) was now turned so that the reduced solution would be forced from C to A.

When A, of about 200 cc. capacity was nearly full, nitrogen was admitted and bubbled

through the solution by way of 5, 4, 6 and 3, escaping through 2 and 1, and mercury trap attached to G. C was now disconnected at H and the tube between H and cock 2 was left full of reduced solution. The material in A, when held between closed cocks, showed no signs of oxidation.

The reduced solution was now moved to the burette B, from which it was measured out into the electrode vessel through K. From a separate outside burette, the oxidized form was added to the reduced, and the E.M.F. was measured on a type K potentiometer. Having obtained a series of E.M.F. readings and a corresponding series of values for (S_r) a curve $\frac{(S_r)}{(S_o)}$

could be plotted representing the complete behavior of the system for the particular pH at which the measurements were made.

This method did not give a satisfactory curve however. As was stated before the author~~y~~ feel that this method can be made to give the best results of any method, when the technical difficulties involved are mastered.

The second method employed, and one

which also gave unsatisfactory results was that of a quinone titration. The apparatus of figure 4 was used to prepare a reduced solution of the methylene blue salt. A measured amount of this solution was then titrated potentiometrically with quinone.

The third method and the one which gave very satisfactory results was the converse of the quinone titration, that is, it was a reduction titration using titanous chloride solution.

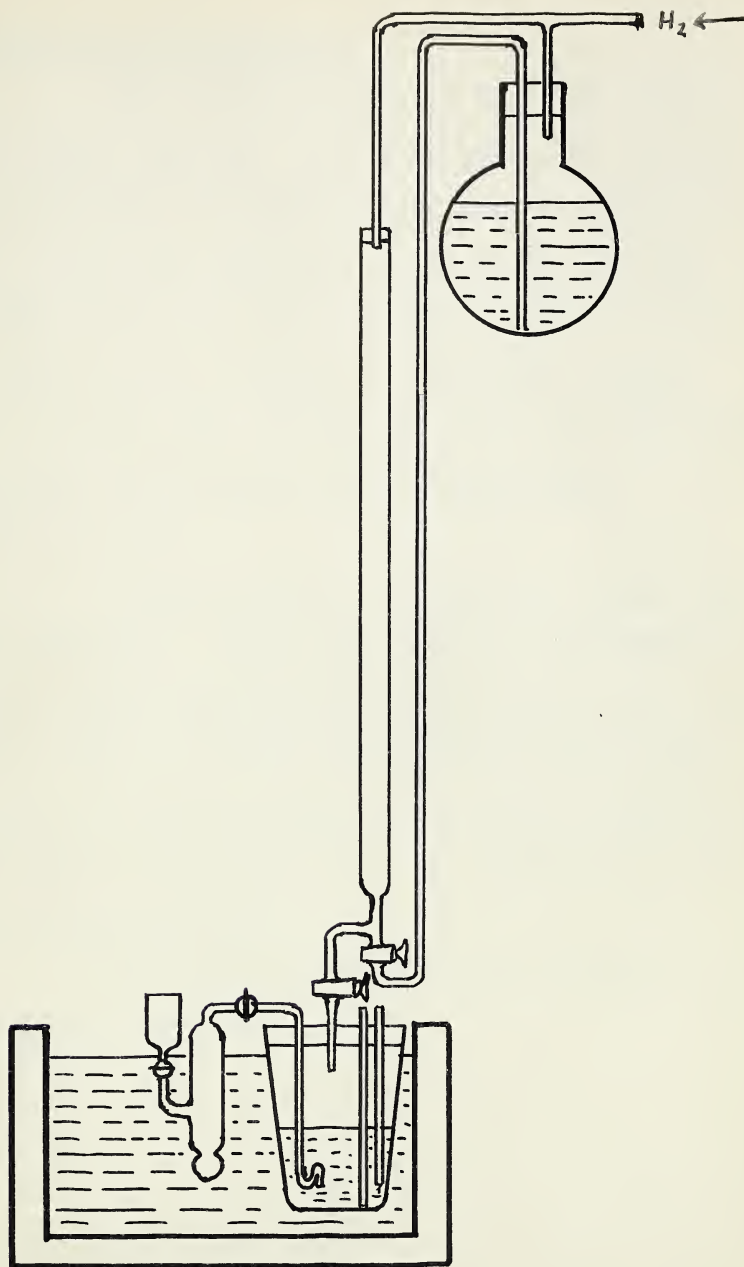
By this method potential readings were made at various steps, during the titration of the oxidized Methylene blue with titanous chloride solution, each being dissolved in buffers, and contained in a protected system. Both visual and graphical end points were taken and in all cases these were very close together. The E.M.F. at the half titrated point was then taken as equal to E_0 .

In some cases, the methylene blue solution was titrated into the titanous chloride solution. When the first permanent blue color appeared the number of c.c. of Methylene blue which had been added was taken as equal to total reductant. As more Methylene blue was added, E.M.F. readings were made until the total oxidant exceeded the total reductant. The value of E_0 was then

read from the point on the curve where total oxidant was equal to total reductant.

It was found that the titanous chloride could be kept in solution at alkaline pH's by the addition of citric acid to the buffers. By this method, titanous chloride solutions down to a pH of 10 were made quite stable. The apparatus used was that which is shown in figure 5. The titanous chloride solution was contained in a hydrogen protected system which held its concentration constant for several hours. The absolute strength of the solution did not matter since the half titrated point was obtained graphically in all cases, and not from calculations of normalities.

Figure 8



Observations and Results.

The following sets of data and graphs were those obtained from E_h measurements during the titration of reduced solutions of methylene blue salts with quinone. It was expected that a curve would have been obtained similar to one of those shown in figure 1. Had such a curve been obtained the inflection point would have represented the E_h value at the point where $(\text{Red}) = (\text{Ox})$.

Figures (a) to (c) show the curves which were obtained. These results were unsatisfactory and no interpretation has as yet been placed upon them. While all three do somewhat resemble the curves of figure 1, they are not reproducible, and the apparent end point does not correspond to the calculated end point.

All such titrations were conducted in an oxygen free system, at 25°C.

Titration of 17.7 c.c. reduced Methylene Blue Thiocyanate
.00009 M with quinone .00009 M at pH of 1.0

E_h	c.c. quinone
.412	1.13
.428	3.09
.436	5.00
.440	6.96
.440	10.00
.440	13.92
.446	17.00
.447	19.06
.447	21.00
.448	25.00

^{sets}
This [^]data is plotted in figure (a).

384

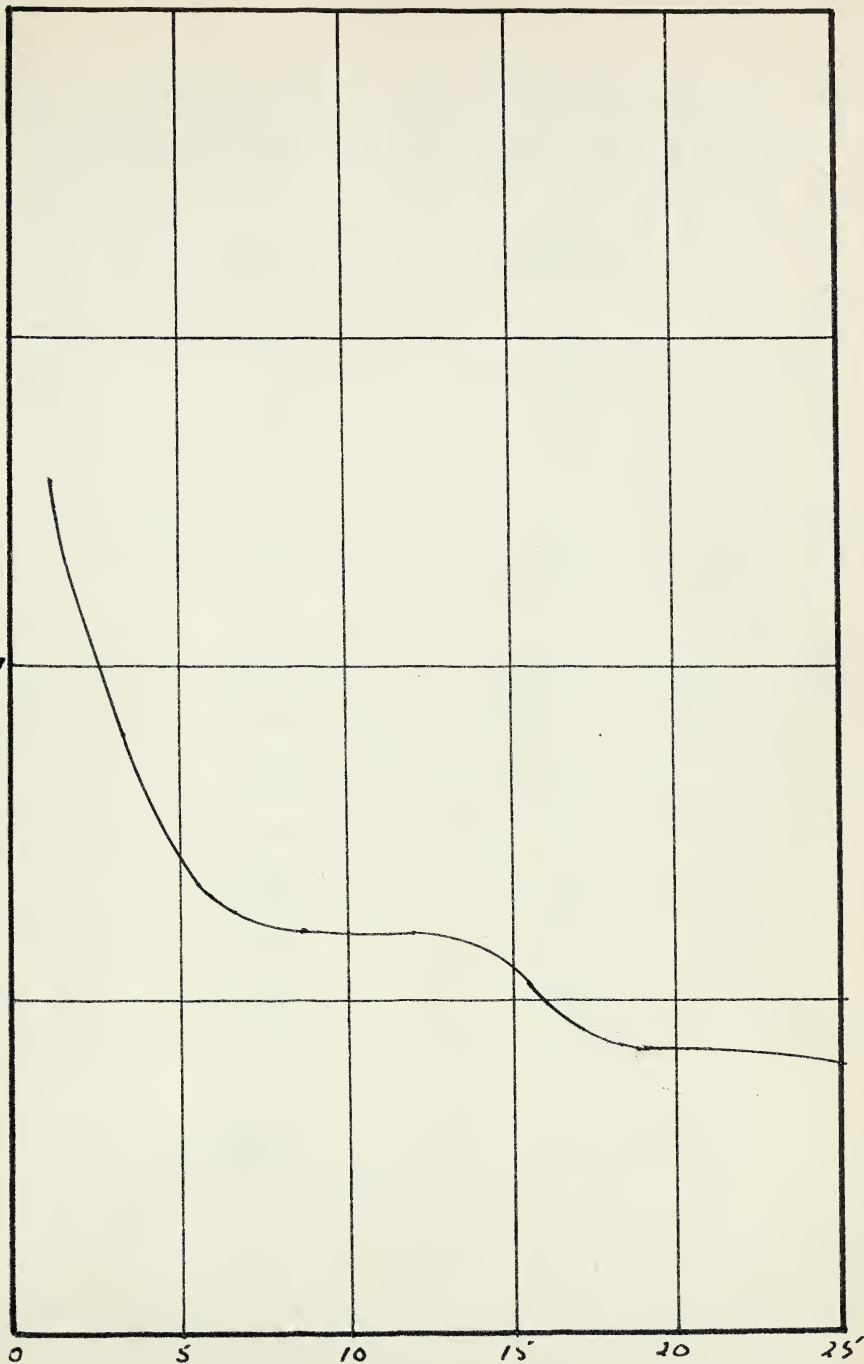
.404

.424

 E_h

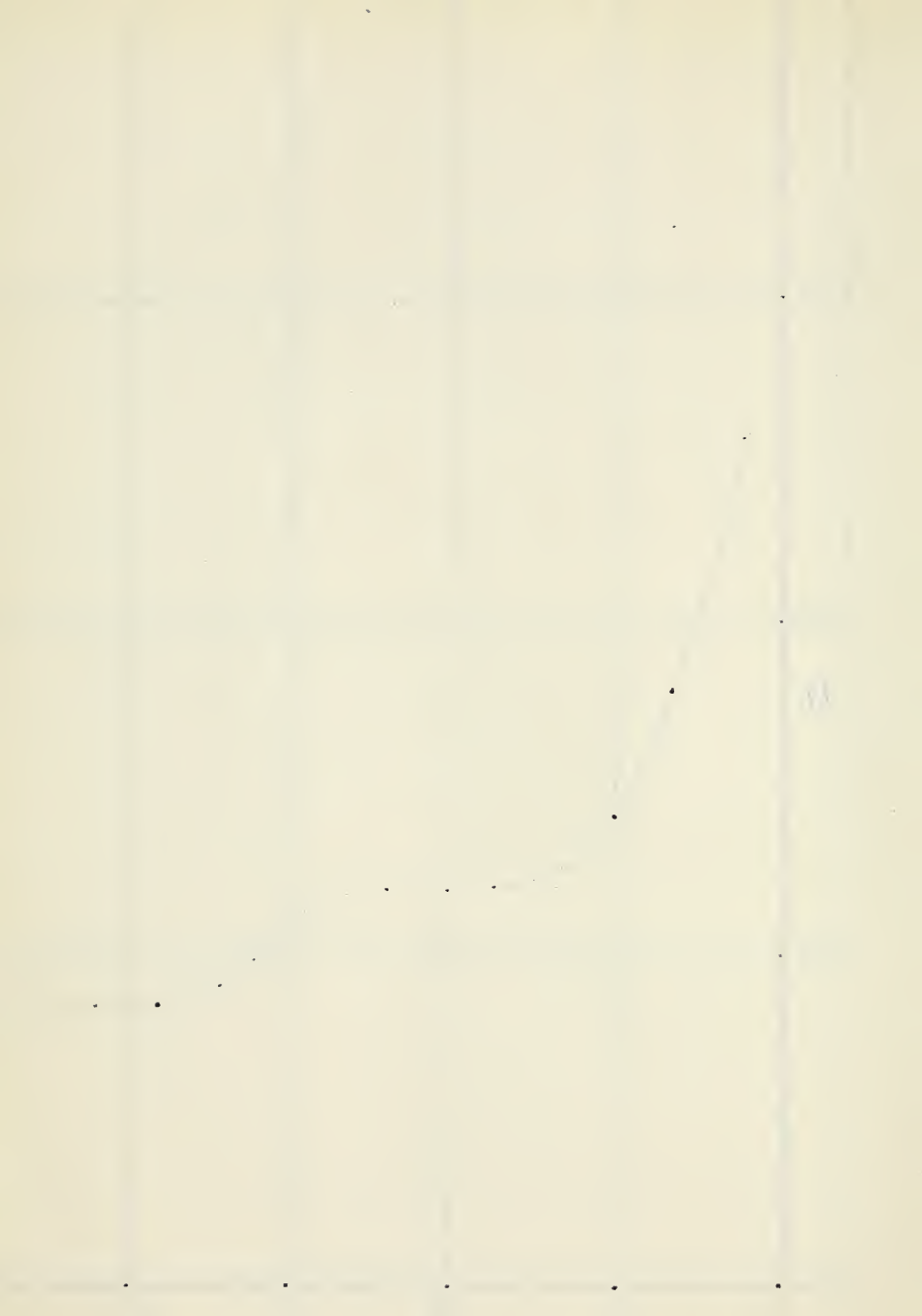
.444

.464



C. C.

Titration curve of reduced methylene blue thiocyanate against quinone at pH of 1.

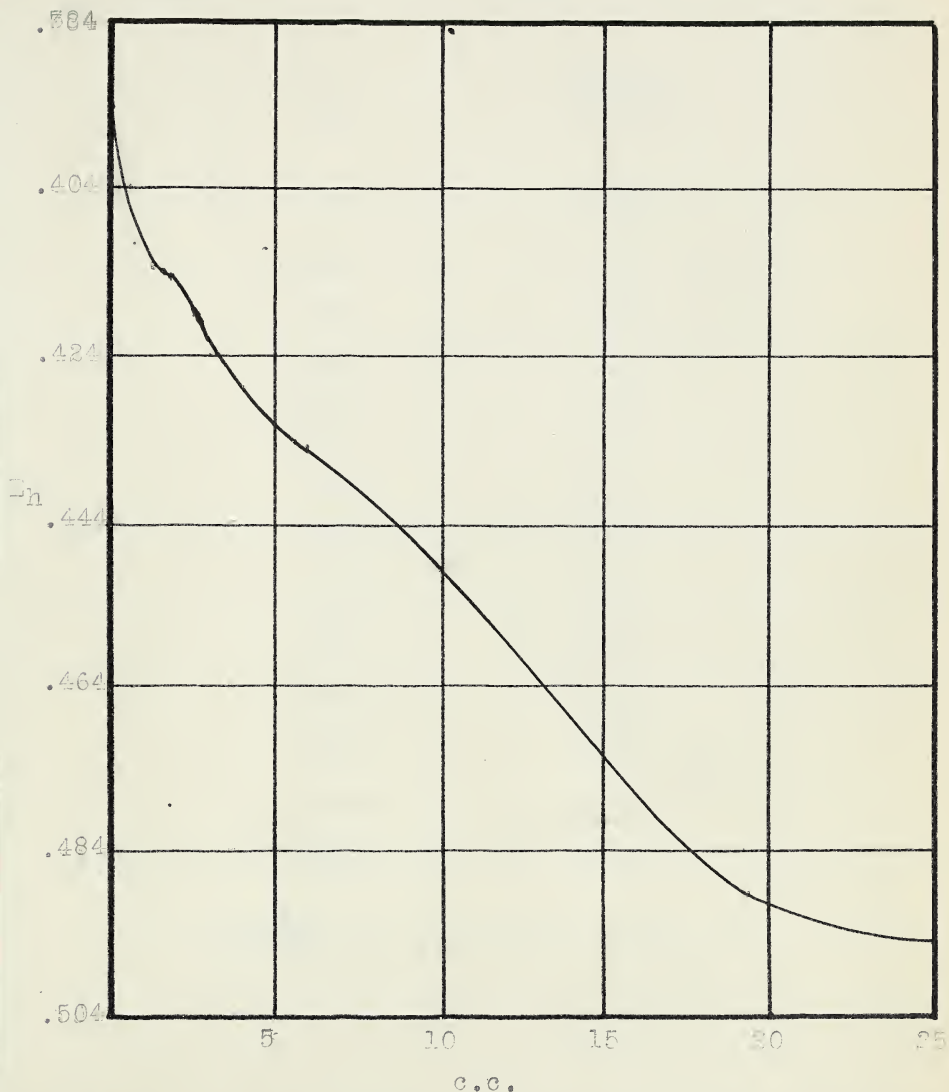


Titration of 17.7 c.c. reduced Methylene Blue Thiocyanate
.00009 M with quinone .00009 M at pH of 1.0

E_h	c.c. quinone
.394	0.1
.404	0.5
.411	0.9
.413	1.3
.415	1.5
.417	2.0
.424	3.0
.428	4.0
.432	5.0
.434	5.6
.435	6.0
.450	10.0
.489	19.0
.495	25.0

^{set of}
This data is plotted in figure (B)

Figure (b)



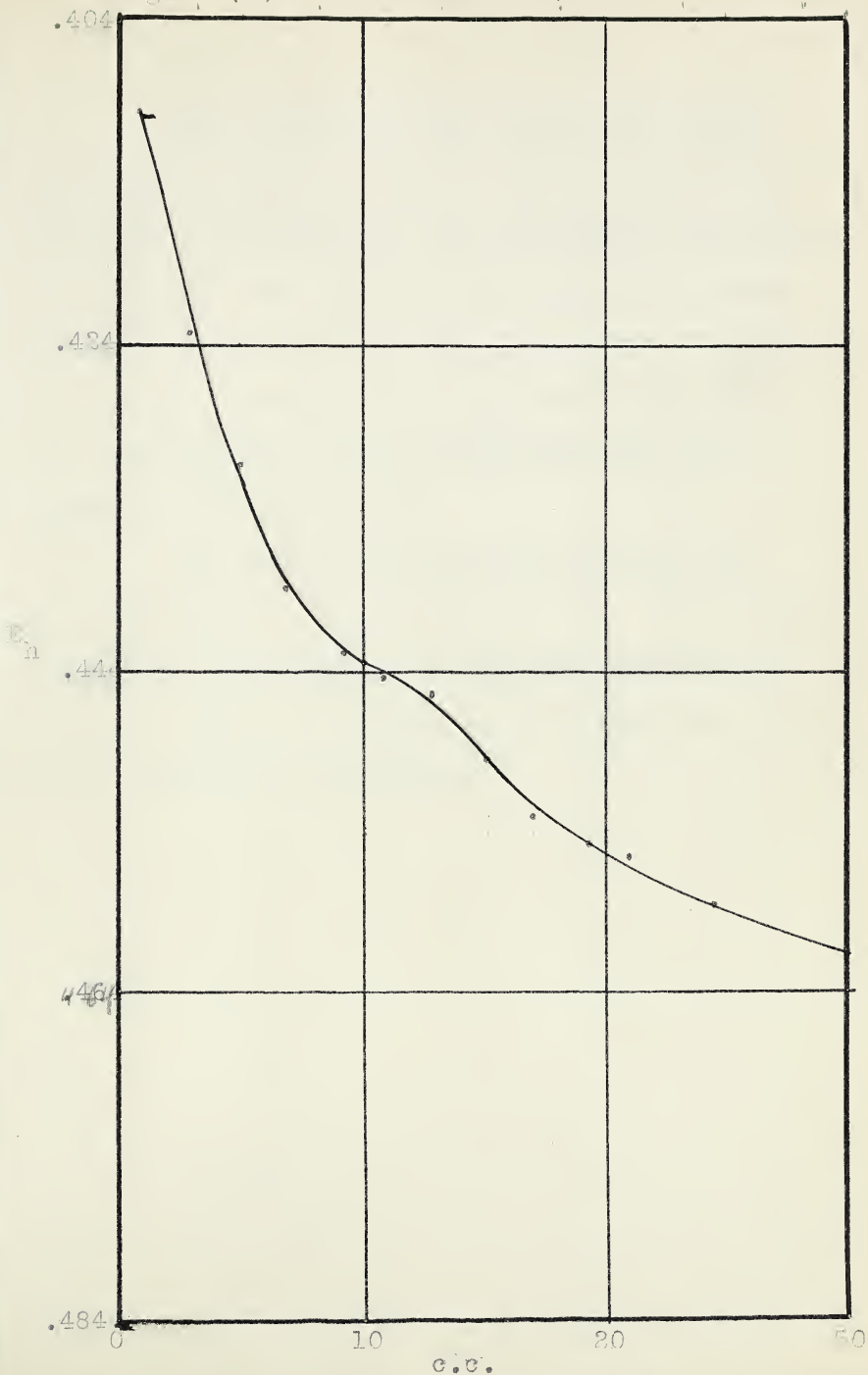
Titration curve of reduced methylene blue thiocyanate against quinone, at pH of 1.

Titration of 20 c.c. reduced Methylene Blue Thiocyanate
.00009 M with quinone .00009 M at pH of 1.0

E_h	c.c. quinone
.410	1.0
.423	3.0
.431	5.0
.439	7.0
.442	9.0
.445	11.0
.447	13.0
.449	15.0
.451	17.0
.455	19.0
.456	21.0
.458	24.0
.461	30.0

^{set of}
This data is plotted in figure (c)

Figure (c)



Titration curve of reduced methylene blue thiocyanate against quinone at pH 7.0

The following four tables and graphs represent attempts to obtain oxidation reduction potentials of methylene blue systems by the method of mixtures. The apparatus of figure 4 was used for these measurements. The system, at all times, consisted of nothing more than simple mixtures of the oxidized and reduced methylene blue salts in buffers at a definite pH.

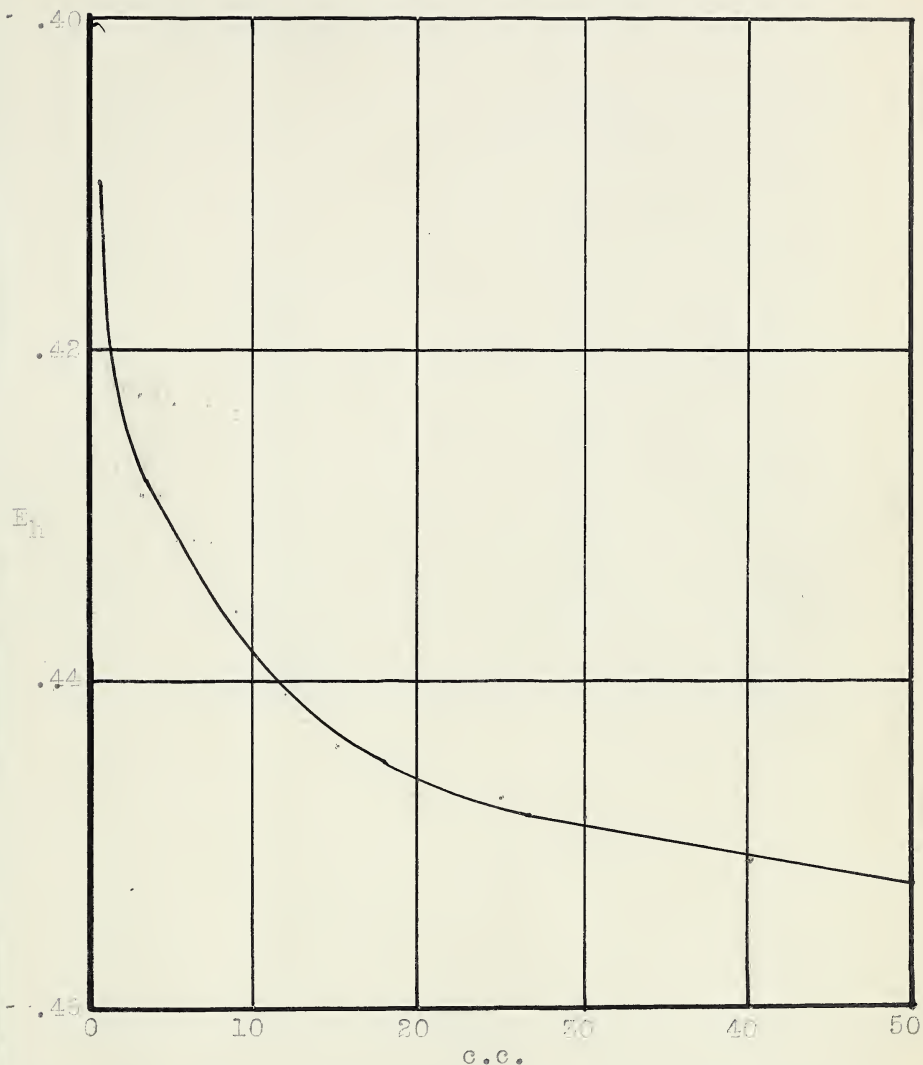
The curves obtained by plotting the data are shown in figures (d) to (g). While these curves are consistently of the same general type they are useless for purposes of calculating oxidation reduction potentials.

Titration of 5 c.c. reduced methylene blue thiocyanate
.0001 M with the oxidized methylene blue thio-
cyanate .0001 M at pH of 1.0.

E_h	c.c. M. B. Ox.
.410	1
.424	2
.429	3
.429	4
.432	5
.432	6
.432	7
.436	9
.441	12
.444	15
.446	20
.447	25
.448	30
.451	40
.452	50

^{at 1.0}
This data is plotted in figure (d)

Figure (d)



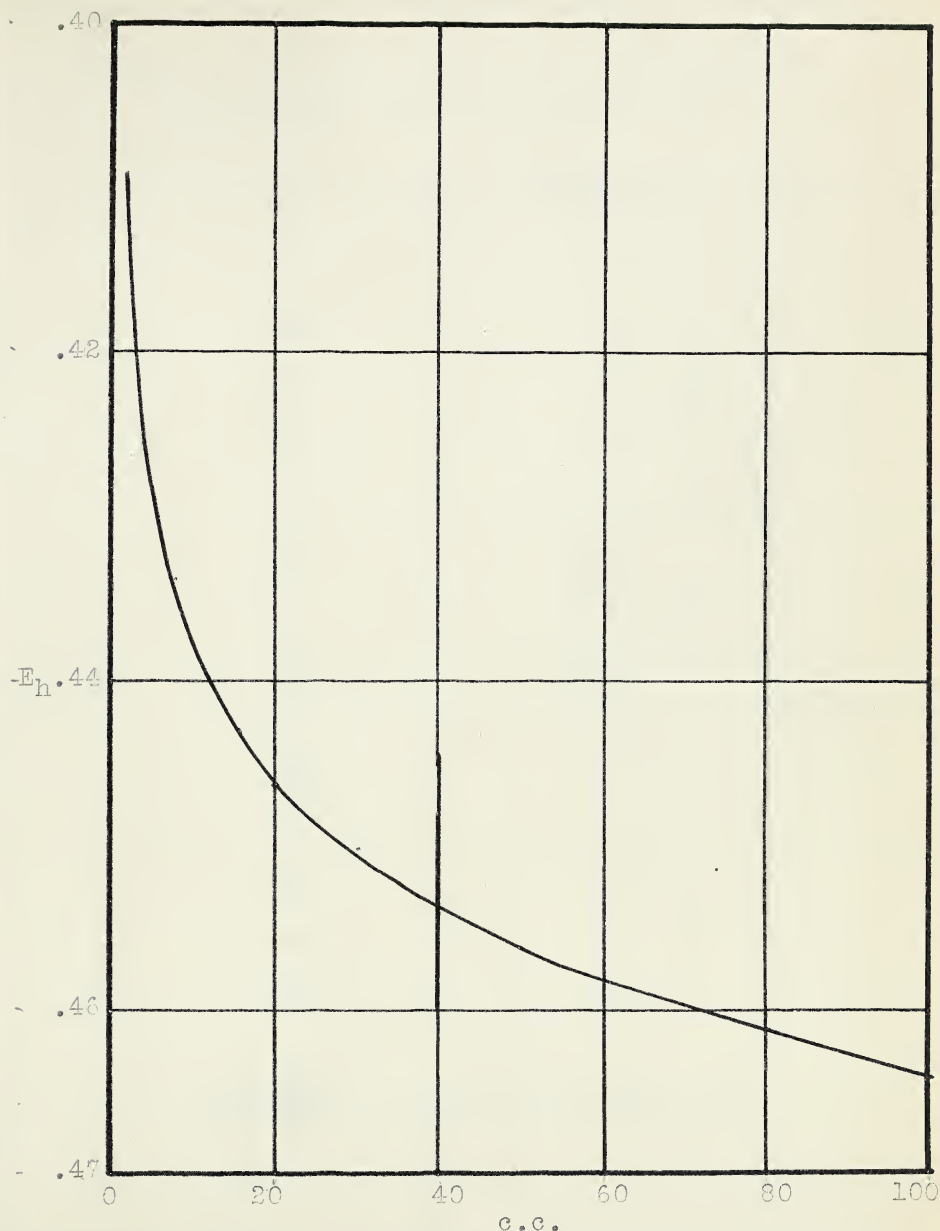
Titration curve of oxidized methylene blue thiocyanate against reduced methylene blue thiocyanate at pH of 1.

Titration of 20 c.c. of reduced methylene blue thiocyanate .0001 M with oxidized methylene blue thiocyanate .0001 M at a pH of 1.0.

E_h	c.c. M. B. Ox.
.409	2
.434	8
.443	16
.446	20
.450	30
.456	50
.461	80
.464	100

This ^{set of} data is plotted in figure (e)

Figure (e)



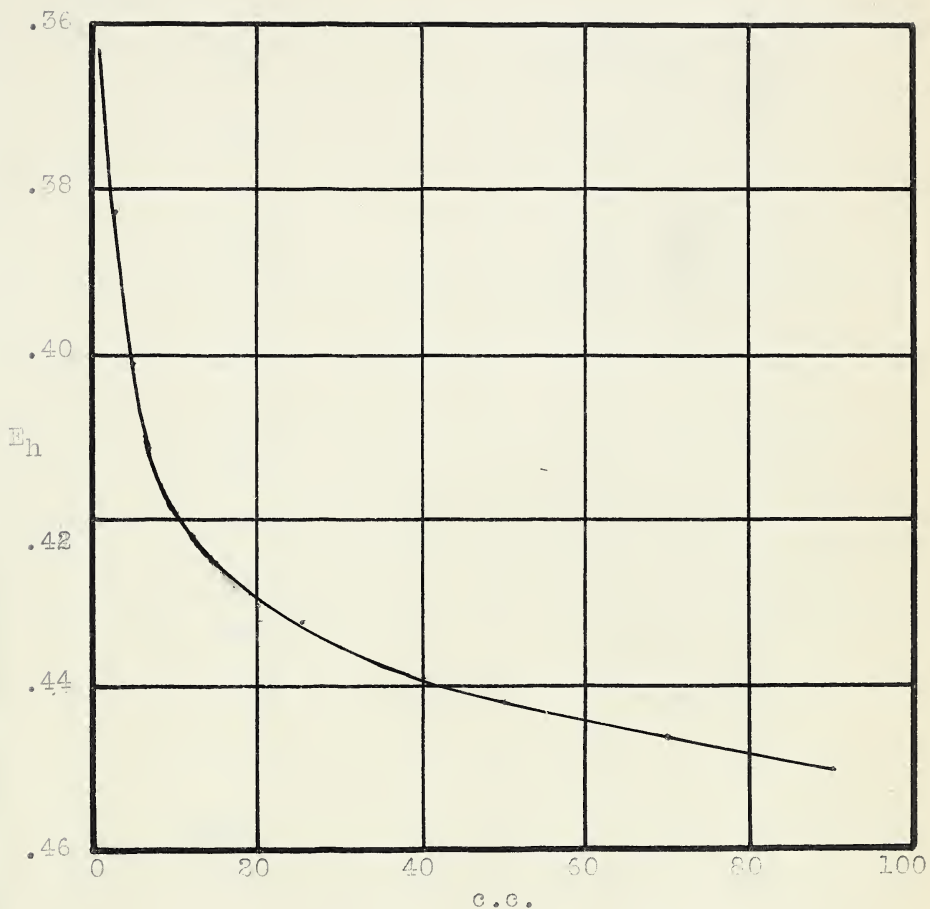
Titration curve of oxidized methylene blue thiocyanate
against reduced methylene blue thiocyanate at pH of 1.

Titration of 80 c.c. of reduced methylene blue thiocyanate .0001 M with oxidized methylene blue thiocyanate .0001 M at pH of 1.0

E_h	c.c. M. B. Ox.
.363	1
.383	3
.401	5
.411	7
.417	9
.419	10
.425	15
.430	20
.432	25
.435	30
.439	40
.442	50
.446	70
.450	90

^{set of}
This data is plotted in figure (f)

Figure (f)



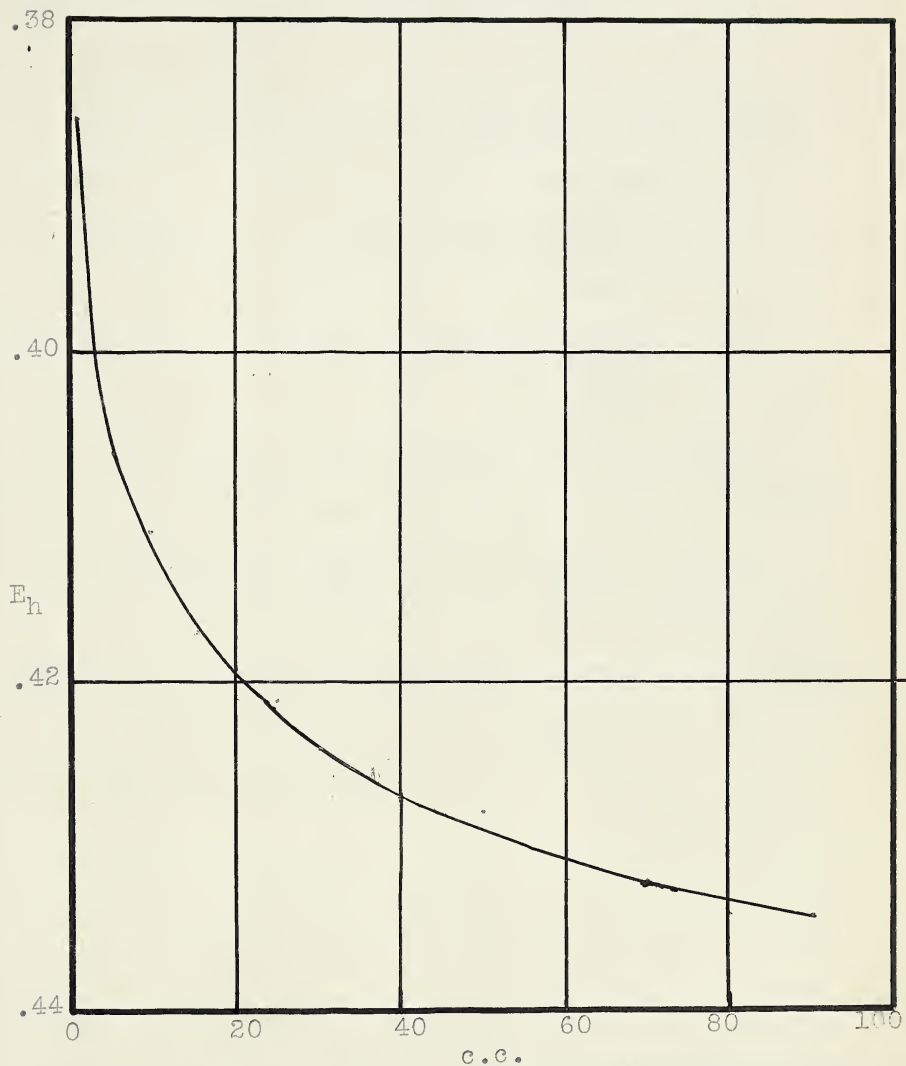
Titration curve of reduced methylene blue thiocyanate against oxidized methylene blue thiocyanate, at pH of 1.

Titration of 100 c.c. of reduced methylene blue chloride .0001 M with oxidized methylene blue chloride .0001 M at pH of 1.0.

E_h	c.c. M. B. Ox.
.386	1
.406	5
.411	10
.413	12
.417	15
.419	20
.421	25
.424	30
.427	40
.428	50
.432	70
.434	90

^{set of}
This data is plotted in figure (g)

Figure (g)



Titration curve of reduced methylene blue chloride against oxidized methylene blue chloride at pH of 1.

The following set of data and graphs were obtained from E_h measurements during the titration of oxidized methylene blue salts with titanous chloride. The stoichemetric end point of such a titration can be obtained from a visual observation as the blue color disappears during the titration, or it can be obtained from the graph. On the graph it is represented by the number of c.c. directly below the middle of the vertical drop in the curve. This point represents 100% reduction of the methylene blue solution. The half titrated point represents the point where (Red) is equal to (Ox) or where $(Red)/(Ox) = 1$

This method gave the most satisfactory results of the three which were tried. The values of E_0 were in most cases the same for both the chloride and thiocyanate.

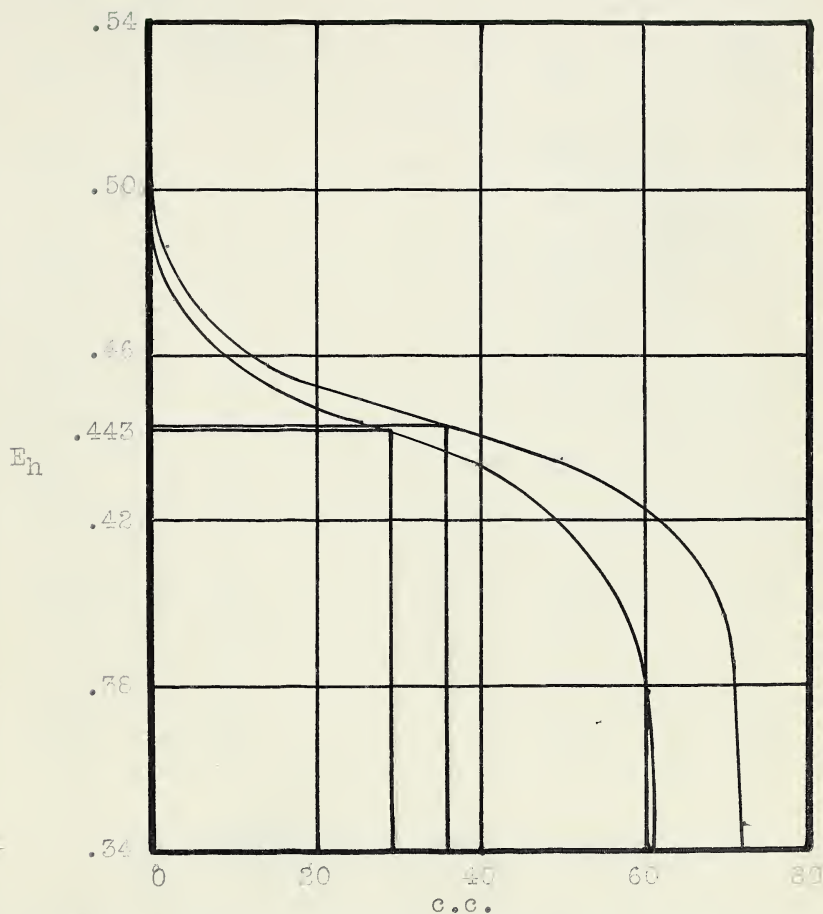
Titration of methylene blue chloride and methylene blue
thiocyanate with titanous chloride solution

.0001 N at pH 1.1.

M. B. Chloride		M. B. Thiocyanate	
E_h	C.C. $TiCl_3$	E_h	C.C. $TiCl_3$
.511	0	.511	0
.486	2	.474	3
.471	6	.464	7
.458	14	.453	15
.450	25	.445	25
.441	40	.434	40
.435	50	.378	60
.425	60	.350	61
.397	70	.335	62
.386	71		
.346	72		

This ^{set of} data is plotted in figure (h)

Figure (h)



Titration curve of methylene blue chloride and thiocyanate against titanous chloride solution at pH of 1.1.

Titration of methylene blue thiocyanate and methylene
blue chloride with titanous chloride solution

.0001 M at pH of 1.3

M. B. Chloride

M. B. Thiocyanate

E_h c.c.
 $TiCl_3$

E_h c.c.
 $TiCl_3$

.447 1

.448 1

.430 2

.439 2

.427 4

.428 4

.412 10

.412 10

.404 12

.407 12

.401 13

.404 13

.396 14

.400 14

.388 15

.395 15

.362 16

.388 16

.350 16.5

.366 17

.342 18.

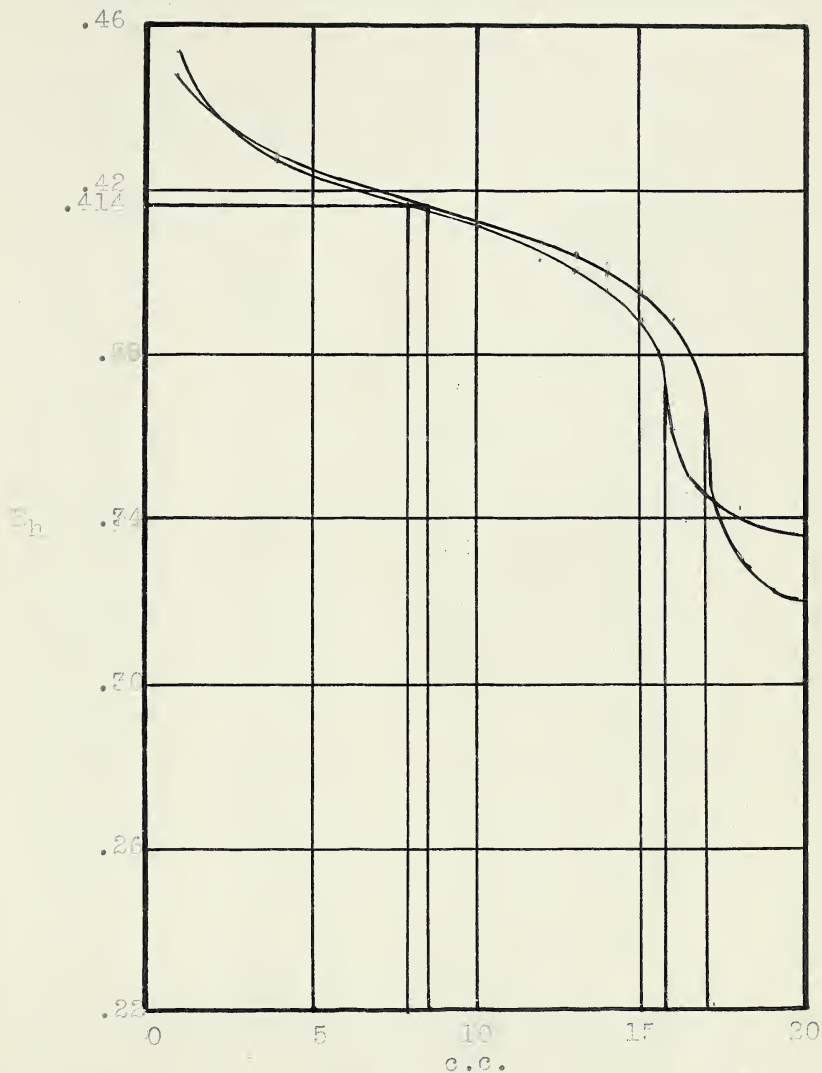
.331 18

.335 20.

.320 20

This ^{set of} data is plotted in figure (i)

Figure (i)



Titration curve of methylene blue thiocyanate and chloride against titanous chloride solution at pH of 1.3

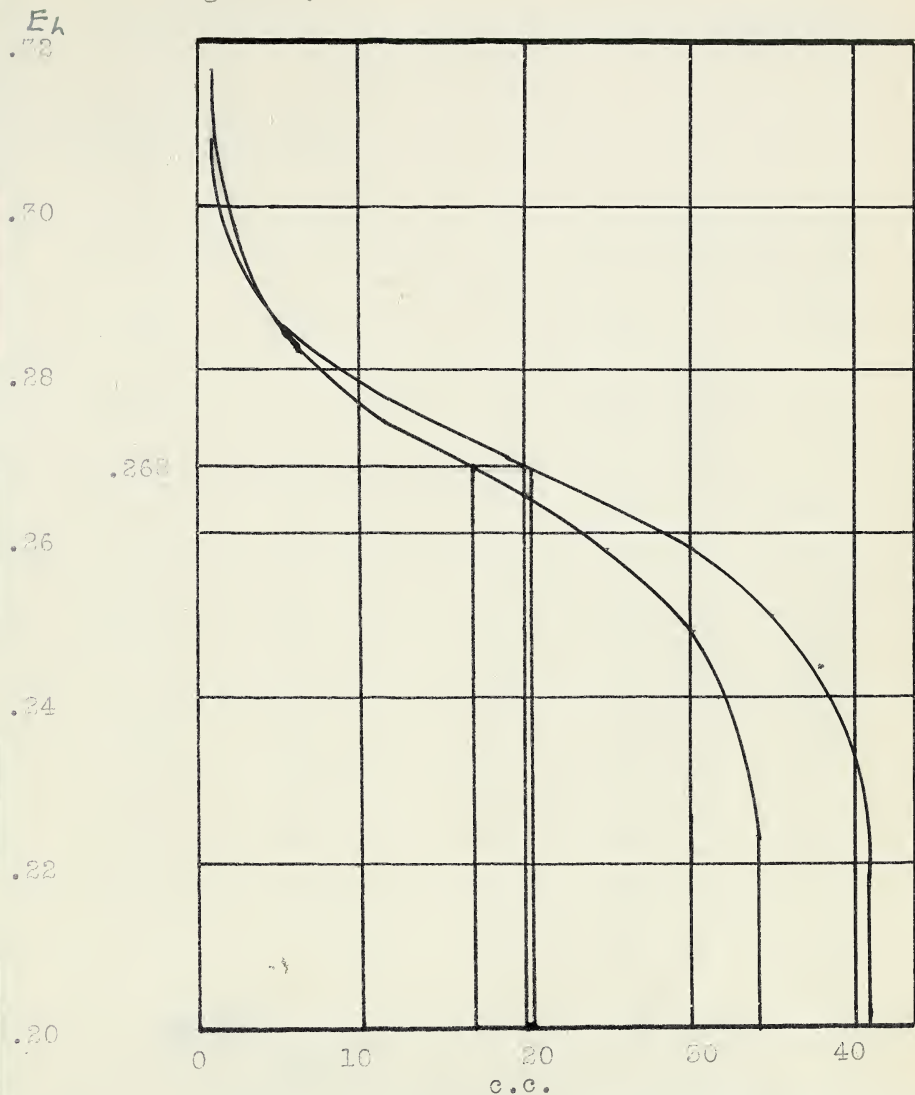
Titration of methylene blue thiocyanate and methylene blue chloride with titanous chloride solution at pH of 3.0.

* Visual end point.

M. B. Chloride		M. B. Thiocyanate	
E_h	c.c. $TiCl_3$	E_h	c.c. $TiCl_3$
.308	1	.317	1
.286	5	.286	5
.279	10	.276	10
.268	20	.269	15
.258	30	.264	20
.250	35	.258	25
.245	37	.254	27
.242	38	.248	30
.236	39	.240	32
*.217	41	*.223	34
		.148	36

This ^{set of} data is plotted in figure (j)

Figure (j)



Titration curve of methylene blue thiocyanate and chloride against titanous chloride solution at pH of 3.



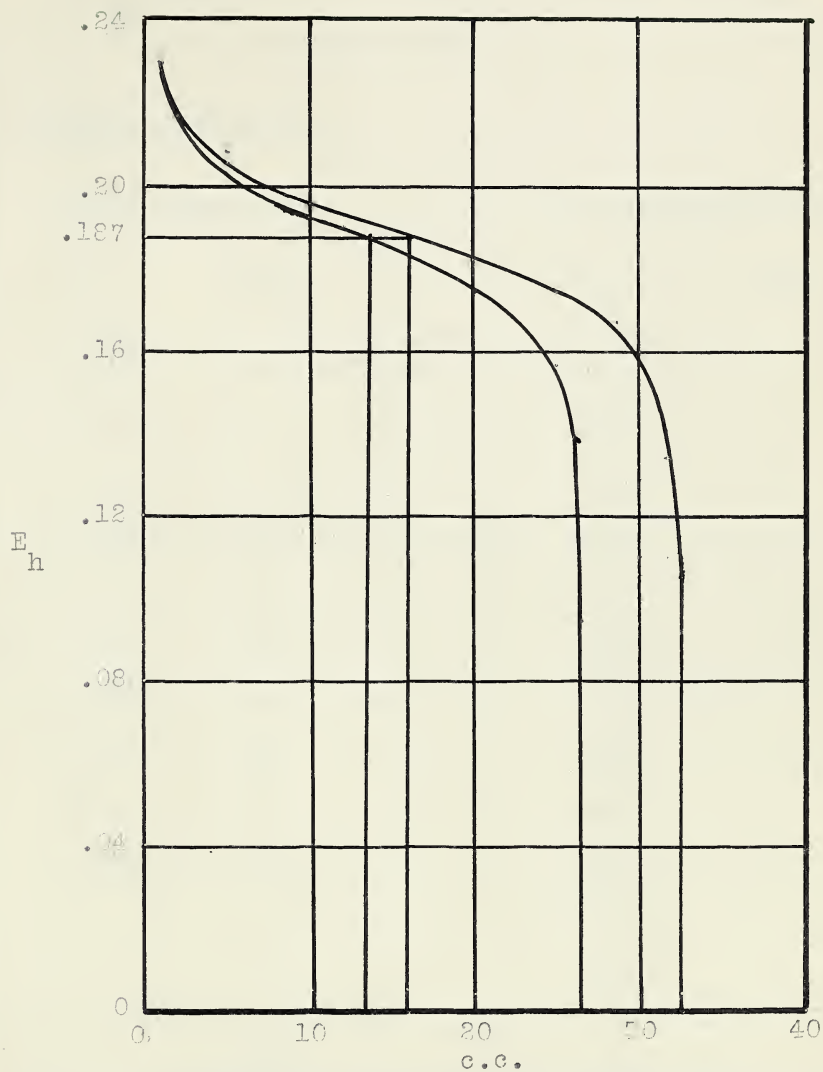
Titration of methylene blue thiocyanate and methylene
blue chloride with titanous chloride solution
at pH of 3.8.

* Visual end point.

M. B. Chloride		M. B. Thiocyanate	
E_h	c.c. $TiCl_3$	E_h	c.c. $TiCl_3$
.229	1	.227	1
.217	2	.217	2
.204	5	.205	5
.190	12	.195	10
.176	20	.182	20
.164	24	.175	25
.158	25	.168	28
.151	25.5	.164	29
.140	26	.160	30
*.095	26.5	.151	31
		*.135	32
		.106	33

This ^{set of} data is plotted in figure (k)

Figure (k)



Titration curve of methylene blue thiocyanate and chloride with titanous chloride solution at pH of 3.8.

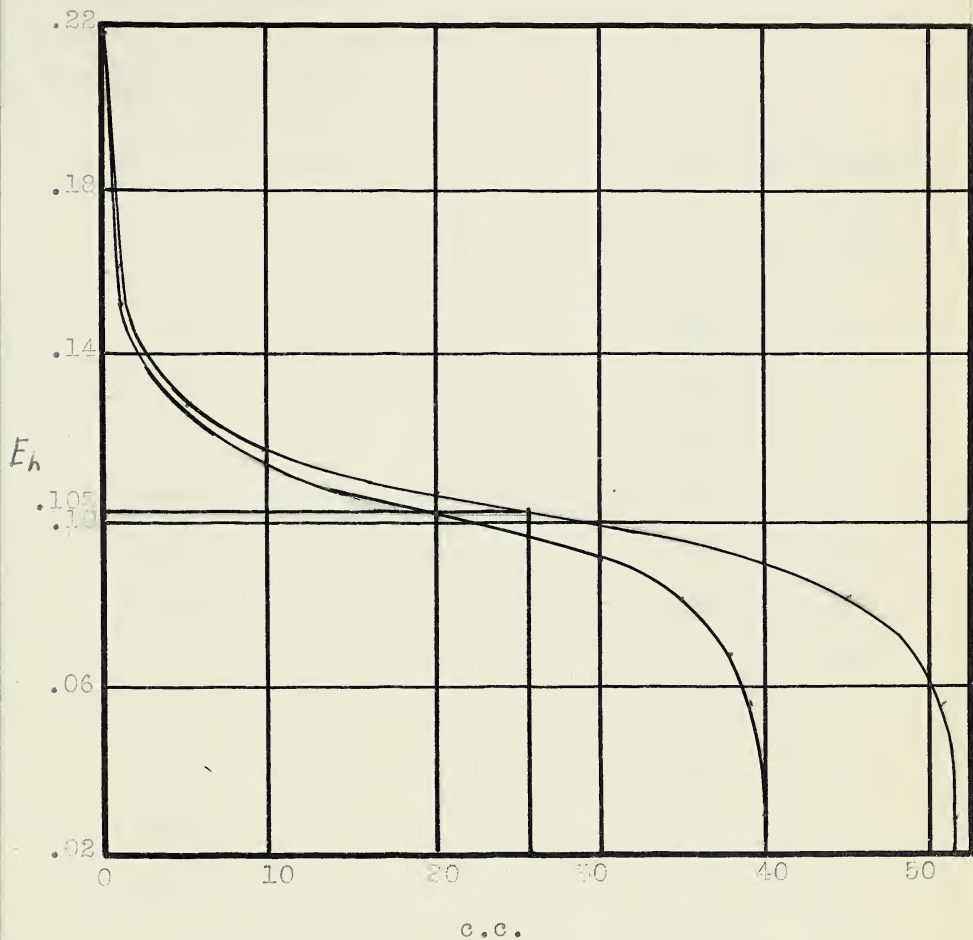
Titration of methylene blue thiocyanate and methylene
blue chloride with titanous chloride solution
at pH of 5.0.

* Visual end point

M. B. Chloride		M. B. Thiocyanate	
E_h	c.c. $TiCl_3$	E_h	c.c. $TiCl_3$
.219	0	.213	0
.153	1	.161	1
.126	5	.128	5
.114	10	.118	10
.102	20	.108	20
.090	30	.099	30
.081	35	.095	35
.067	38	.090	40
*.056	39	.082	45
.030	40	.065	50
		*.055	51
		.030	52

This ^{set of} data is plotted in figure (1)

Figure (1)



Titration curve of methylene blue thiocyanate and chloride against titanous chloride solution at pH of 5.0.

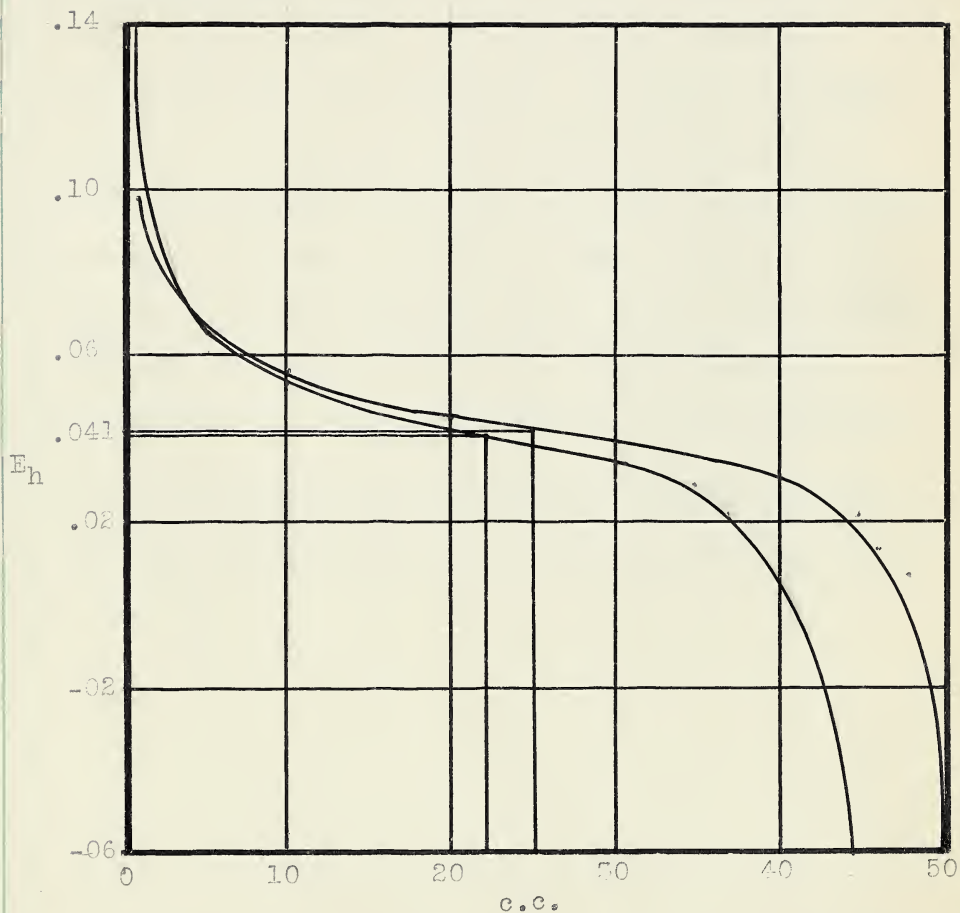
Titration of methylene blue thiocyanate and methylene
blue chloride with titanous chloride solution
at pH of 6.2.

* Visual end point.

M. B. Chloride		M. B. Thiocyanate	
E_h	C.C. $TiCl_3$	E_h	C.C. $TiCl_3$
.143	1	.098	1
.065	5	.068	5
.053	10	.056	10
.041	20	.045	20
.033	30	.039	30
.032	33	.038	35
.031	34	.031	40
.029	35	.026	43
.024	36	.021	45
*.022	37	.013	47
-.060	44	*.007	48
		-.063	50

This ^{set of} data is plotted in figure (m)

Figure (m)



Titration curve of methylene blue thiocyanate and chloride against titanous chloride solution at pH of 6.2.

The following two curves were obtained by a slightly different procedure from that used before. A solution of titanous chloride was added to the electrode vessel, and methylene blue solution run in from a burette until the first permanent blue color made its appearance. It was now assumed that all the methylene blue which had been run in was in the reduced form, and that this quantity could be used as a measure of (Red). More oxidized methylene blue solution was now run in and E_h measurements were made at various intervals. When plotted as E_h against c.c., the point where the excess oxidized solution was equal to the original reduced solution was taken as the point where $(\text{Red})/(\text{Ox}) = 1$ and where $E_h = E_o$.

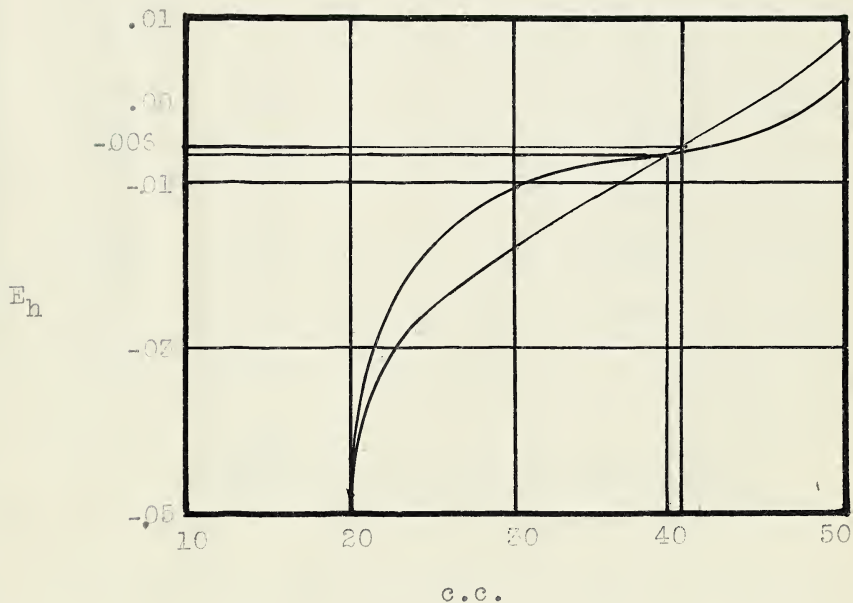
Titration of a titanous chloride solution with methylene blue thiocyanate solution and with methylene blue chloride solution at a pH of 8.0.

* Visual end point.

M. B. Chloride		M. B. Thiocyanate	
E_h	c.c. M. B.	E_h	c.c. M.B.
	*20.0		
-.042	20.8		*19.5
-.031	22	-.048	20
-.028	24	-.011	30
-.018	30	-.007	40
-.006	40	+ .002	50
+ .008	50		

This ^{set of} data is plotted in figure (n)

Figure (n')



Titration curve of titanous chloride solution against methylene blue thiocyanate and chloride solutions at pH of 8.0.

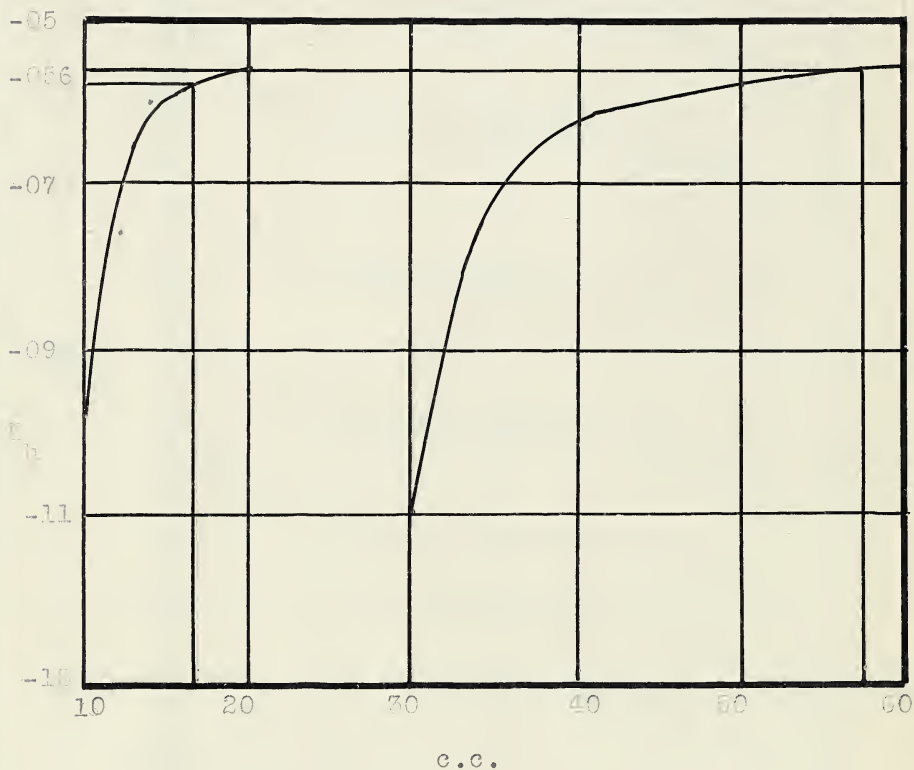
Titration of a titanous chloride solution with methylene
blue thiocyanate solution and with methylene
blue chloride solution at a pH of 9.

* Visual end point.

M. B. Chloride		M. B. Thiocyanate	
E_h	c.c. M.B.	E_h	c.c. M.B.
-.114	*8.4	-.134	*28.7
-.098	9.0	-.110	30.0
-.076	12.0	-.063	40.0
-.060	14.0	-.057	50.0
-.059	16.0	-.055	60.0
-.056	20.0		

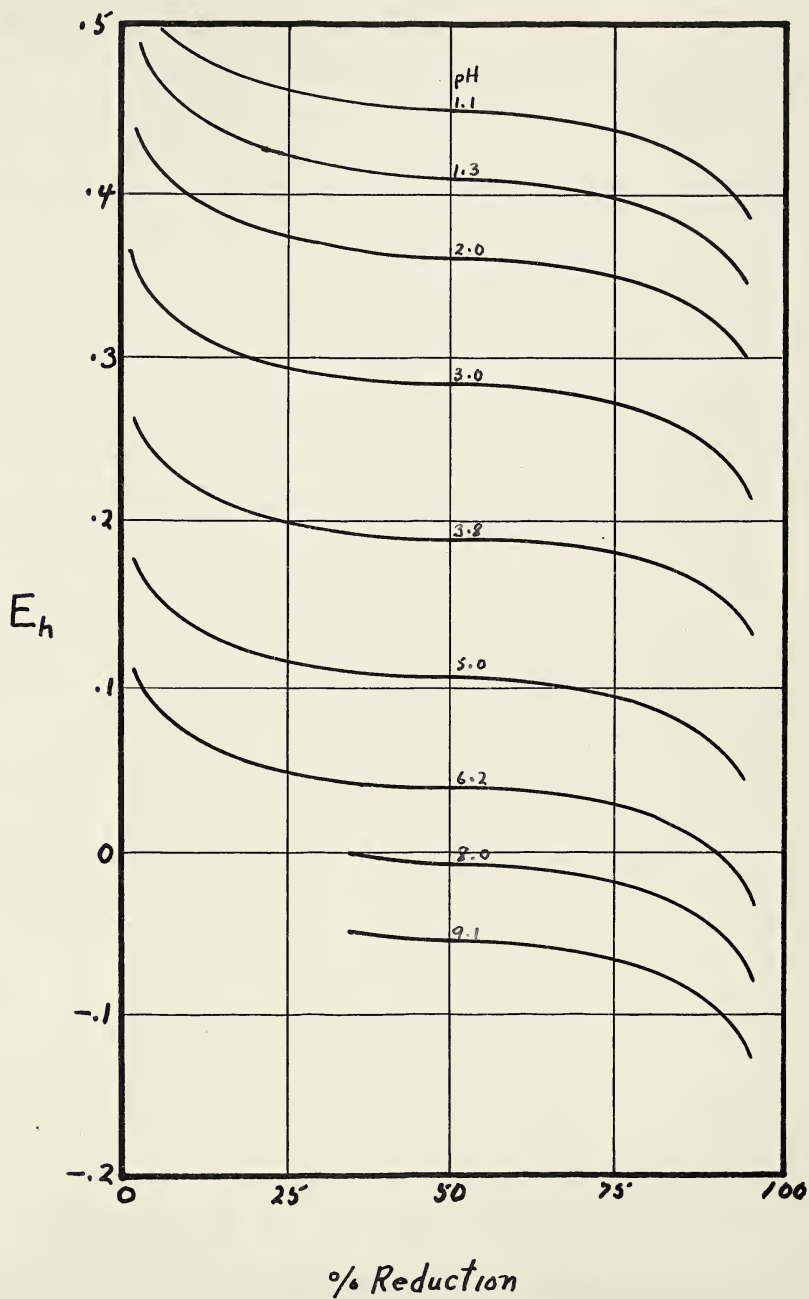
^{set of}
This data is plotted in figure (0)

Figure (o)



Titration curves of titanous chloride solution against methylene blue thiocyanate and chloride solutions at pH of 9.1.

Figure 6



Summary of the Observations

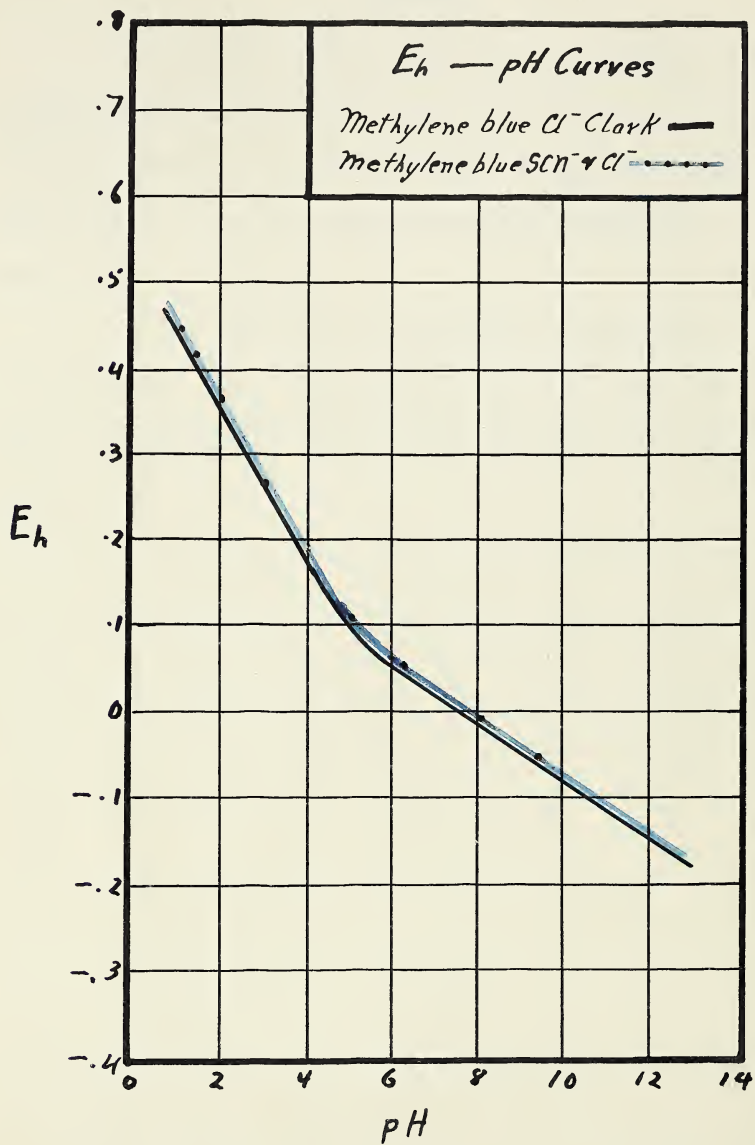
If the curves from (h) to (o) be plotted on one graph as E_h against percentage reduction, each pair will be represented by a single curve as shown in figure 6.

The following table shows the relation of E_h to pH, and the corresponding graph is plotted in figure 7. This curve agrees well with both the experimental curve obtained by W. M. Clark and with the theoretical analysis given before.

E_h	pH
.443	1.1
.414	1.3
.383	2.0
.268	3.0
.187	3.8
.103	5.0
.041	6.2
-.006	8.0
-.056	9.1

*Relation of E_h to pH when (Red)=(Ox)
for both methylene blue thiocyanate and
methylene blue chloride.*

Figure 7



Summary

The oxidation-reduction potentials of the system methylene blue thiocyanate have been run over a complete range of pH and have been found to check satisfactorily with the work of W. M. Clark.

The oxidation-reduction potentials of methylene blue thiocyanate are the same as those of methylene blue chloride under the same conditions.

References

1. Thornton, H. R. and Hastings, E. G.
Journal of Bacteriology, Vol. XVIII, No. 5, 1929.
2. Thornton, H. R., J. Dairy Sci. 17: 210-232, 1935.
3. W. M. Clark,
Studies on Oxidation-Reduction,
Hygienic Laboratory bulletin No. 151, page 187.
4. Thornton, H. R. and Hastings, E. G.
Journal Dairy Science 13: 221-245, 1930.
5. Thornton, H. R., Sandin, R. B., Miller, C. S.,
Canadian Journal Research, B. 13: 257-263, 1935.
6. Drew, H. D. K. and Head, F. S. H., J. Chem. Soc.
248-253, 1933.

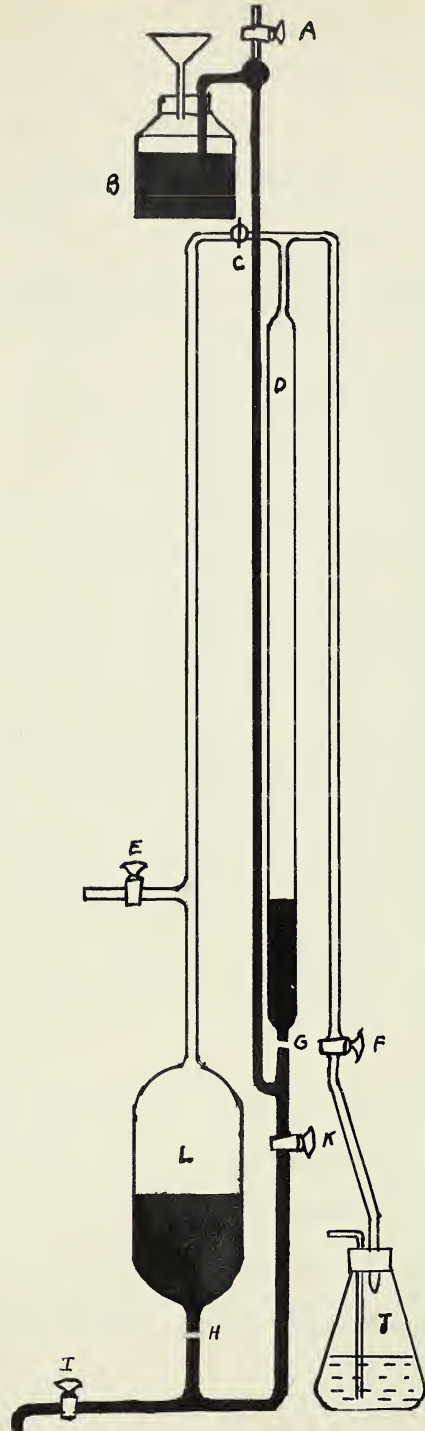
A New Protected Burette for Standard Reducing Solutions

The apparatus used for holding and measuring out the titanous chloride solutions, in the investigation of the oxidation-reduction potentials of methylene blue, is shown in figure 5. While this apparatus was satisfactory for the purposes to which it was put in that work, it is most unsatisfactory for general quantitative analysis.

It has been found that with the best of materials and using the purest of hydrogen, and keeping the system under a positive pressure of hydrogen at all times, still oxygen of the air leaks into such a system and the strength of the reducing solution will not remain within analytical specifications for more than a few hours. In order to obtain a burette which would hold the concentration of such solutions constant over long periods of time, the one shown in figure 8 was designed, using mercury instead of hydrogen to fill all the dead spaces. While the design is original the principle is not new. McFarlane has applied the same principle in the case of a microburette.(1)

The burette is so designed that it can be made in two pieces and put together with rubber tubing. At no point in the system does the

Figure 8



reducing solution come in contact with any material other than glass, or mercury.

The mechanical principle of the apparatus is as follows. A storage reservoir of mercury B is placed above the rest of the system, and from it mercury is siphoned over, creating a head of pressure at G. When cocks C and K are closed and F opened, mercury flows from B to D and the volume which passes into the burette D is equal to the volume of standard solution which flows out of D through F into the titration vessel J. The bulb L is a storage vessel in which the reserve standard solution is stored. This bulb is filled with standard solution through E while mercury passes out through I, and K remains closed. The burette D is filled by opening both K and C while F remains closed. Mercury flows from D to L by way of K and standard solution flows from L to D by way of C.

The apparatus is set up as shown, and solution of titanous chloride in hydrochloric acid is run into L. All air is flushed out through F, and when the system has stood for a day the solution is standardized. It is restandardized at

intervals over a period of a month and a half during which time there is no appreciable change in concentration.

The method of standardization is as follows. A sample of $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ of about one gram, is weighed out and dissolved in H_2SO_4 . This is then titrated with N/10 $\text{K}_2\text{Cr}_2\text{O}_7$ to an accurate end point. After boiling to expel dissolved oxygen, the solution is cooled and an excess of KCN added as an indicator of ferric ions. The vessel is now placed in position J and with a stream of purified nitrogen passing through it, it is titrated to a colorless solution with the titanous chloride solution. The observations are shown in the following table.

Date	Wt. Sample	c.c. TiCl_3	Normality
Feb. 17/36.	1.3198	25.57	.1317
	1.7829	34.60	.1314
	1.0862	21.13	.1312
Mar. 2/36	1.2867	25.01	.1313
	1.6414	31.94	.1314
April 2/36	1.5037	29.25	.1311
	1.6726	31.92	.1306

Conclusion

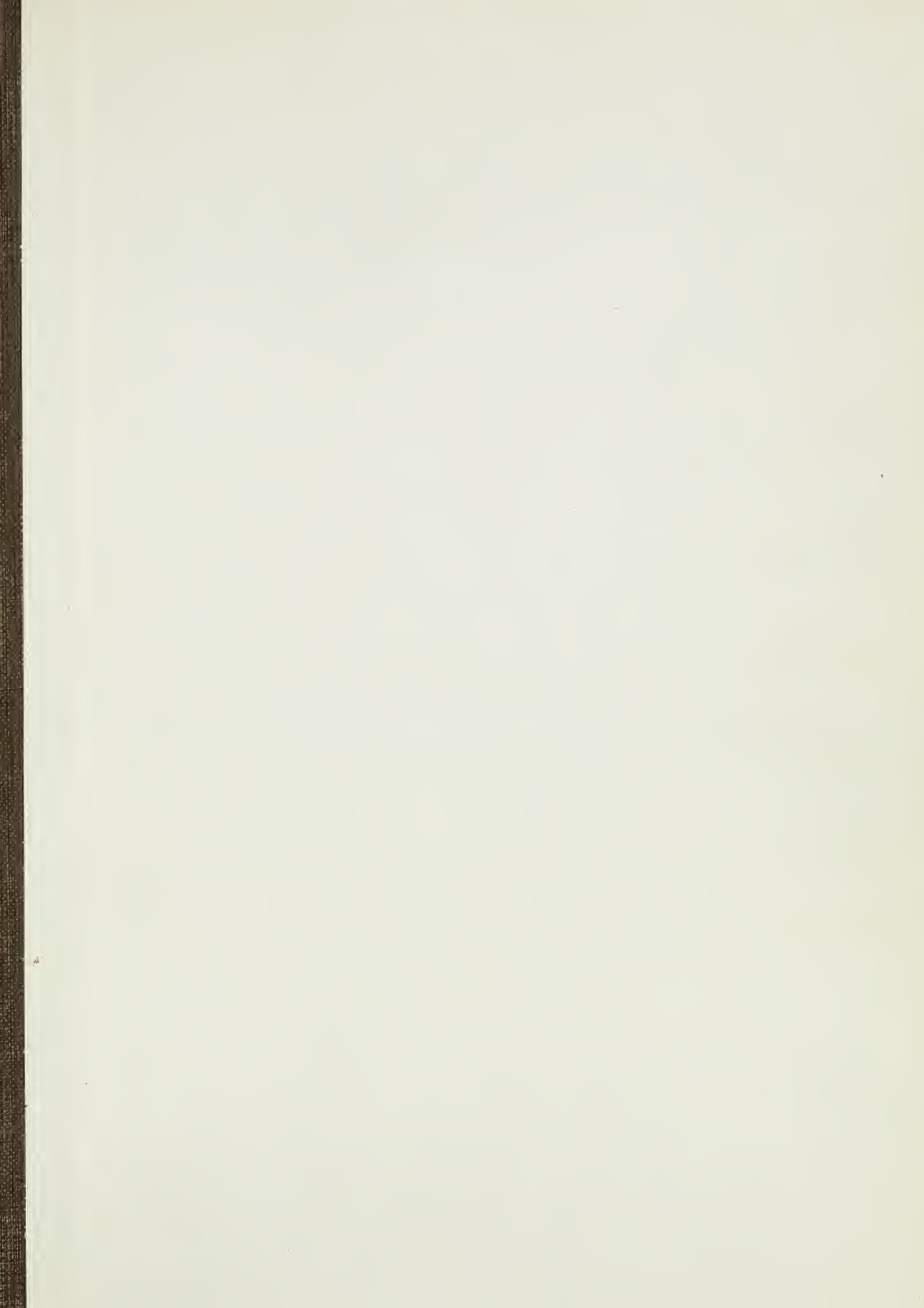
A new burette has been designed and tested which will preserve standard reducing solutions, indefinitely. The apparatus is simple and easy to make.

References

W. D. McFarlane J. Ind. Eng. Chem.

Anal. Ed. Vol 8: No. 2, 124-126, 1936.





B29744